Lesser Studied Pathologies Webinar - January 5, 2024

ME/CFS Research Roadmap Webinar Series – Lesser Studied Pathologies
Open Session
Friday, January 5, 2024

Vicky Whittemore: All right. Good morning, everyone. I'm Vicky Whittemore. I'm a program director at the National Institutes of Neurological Disorders and Stroke and oversee the ME/CFS grant portfolio here and work together with my colleagues across NIH. So, it's my pleasure to welcome you to the seventh of eight webinars that we've held as part of this ME/CFS Research Roadmap Initiative to identify research priorities for ME/CFS.

So, I'd first like to acknowledge the Working Group of Council who are listed -- individuals who are listed here that cross investigators, clinicians, individuals with lived experience, and patient advocates. And we're very thankful for all of them who have served on this and continue to serve on this Working Group of Council. So, in particular, I would like to thank Beth Pollack from MIT, who is the chair -- excuse me -- of this webinar planning group and the other members of this specific planning group who have worked together to put the webinar that you will hear today, together for us.

And I would also like to acknowledge my NIH team, Rebekah especially, who's been working with us a lot for -- with the patients or people with lived experience, as well as James Taylor who's really been helping me behind the scenes to really help to maneuver all of these webinars. And a special shout-out to Holly Riley and Damon Kane at RLA for all of their logistical support also for these webinars.

So, we have one webinar left after today, which will take place on January 11th, on circulation. For more information about the Roadmap Initiative, you can go to the -- if you go to the NIH website, specifically to the NINDS website, and just type in ME/CFS Working Group of Council, it will take you to this link. All of the videos and transcripts from past webinars are posted on the Research Roadmap website. So, you can access all of those -- the previous webinars there. And the current webinar and the one on the 11th will also be posted there when those are ready to be posted.

So, just some guidance for webinar participants. So, the goal of the webinar is to identify research priorities, and this -- in this particular case, on lesser studied pathologies of ME/CFS. So, we've asked the speakers to address what do we know, what don't we know, and what do we need to know to accelerate research to get a better understanding of the physiology in these lesser study pathologies of ME/CFS to help identify targets for treatment.

So, I would ask those questions for the speaker. You may put questions for them in the Q&A, and we will address questions for each speaker after their talks. We're not able to answer any

personal, medical, or health issues or questions that you may have. And questions should focus on clarifying points from the presentations and with regard to research priorities on this topic. Other questions about NIH, ME/CFS research, or other funding or NIH-related issues should be addressed to this email address: nindspublicinquiries@ninds.nih.gov.

So, all of the research priorities from all the webinars will be used to form a report to the NINDS Advisory Council and NINDS leadership that will be presented at the meeting in May of 2024. You can send emails to this email address here, mecfsresearchroadmap@ninds.nih.gov, as well if you have specific comments about the webinar or the research priorities. The best way to receive announcements and updates from NIH is to sign up for the NIH ME/CFS listserv at this URL, so www.nih.gov/mecfs.

And we will be soliciting information and feedback from the community on the research priorities identified during the webinars on a platform called IdeaScale, a crowdsourcing platform. We're just about ready to launch that. So, watch for emails coming out announcing that and giving instructions about how to access and utilize that platform.

So, with that, it's my pleasure to introduce Beth Pollack, who is the chair of this webinar planning group. She's a research scientist in the Department of Biological Engineering at Massachusetts Institute of Technology. And with that, I will turn it over to you, Beth. Thank you.

Beth Pollack: Thank you, Vicky. One second. Okay. Hi, everyone. And welcome to the Less Studied Pathologies webinar. On behalf of the Less Studied Pathologies subgroup, thank you so much for joining us today. My name is Beth, as Vicky mentioned. I have the distinct honor of being a Chair of the subgroup. I am a research scientist at MIT, based in the Tal Research Group, which is an immunoengineering lab studying infection-associated chronic illnesses, which is led by Mikki Tal.

In this introductory talk, I'm going to discuss three main things. One, an overview of what we'll be discussing in our research webinar today. Two, an overview of some of the rates of comorbidities between ME/CFS and some of the pathologies discussed in our webinar with a focus on our first two topics. And three, a brief discussion of examples of how we might incorporate some of these topics into research. So, I have no disclosures.

I'm going to overview the six topics of the webinar. But before I do, I want to share that when many of us joined this greater Research Roadmap Working Group a year ago, the webinar wasn't originally part of the series. And after months of conversation and thinking deeply and comprehensively about important aspects of ME/CFS, it became clear to us that there was a number of less studied pathologies that greatly impact many patients' quality of life and may contribute to illness severity. Thus, we developed the subgroup and this new webinar.

Importantly, I want to note that all of ME/CFS can be considered less studied, especially considering its disease burden. But in this webinar, we discuss emerging topics that have significant impacts on large subsets of ME/CFS patients and that may have many outstanding questions. Many of the speakers here today are clinicians because they are on the front lines, seeing, studying, and trying to treat these pathologies. I also want to note that there are other pathologies that we could have included. And while we aim to cover many of the topics in this webinar not also covered by other webinars in the series, we obviously couldn't cover them all.

The six core topics in ME/CFS covered by our webinar include connective tissue disorders, spinal and mechanical conditions, mast cell activation disorders, gastrointestinal conditions and neurogastroenterology, neuroendocrine dysfunction and sex hormones, and reproductive health conditions, including irregular menstruation, endometriosis, pregnancy, menopause, and men's reproductive health.

This webinar is in some ways an important event because, to my knowledge, this is the first time that some of these pathologies, like connective tissue issues, spinal conditions, and possibly an in-depth discussion of reproductive health at ME/CFS have been discussed at an NIH event or a similar high-profile event in ME/CFS.

Vicky covered these guiding questions, so I will go to my next slide. ME/CFS shares profound

rates of comorbidity with a number of similar and overlapping illnesses. It is important to note that many of these illnesses also share several of the less studied pathologies that we are discussing here today, which I'll describe in the following slides and in my next talk.

There are many unanswered questions about the mechanisms driving and contributing to several of these pathologies. But the fact is that they are shared by subsets of patients across this group of illnesses, many of whom have multiple of these comorbidities, which indicates a need for further research on this topic. Why these illnesses so frequently co-occur and how they overlap, and better understanding the subsets that develop some or multiples of these less studied pathologies and comorbidities is part of what I study at MIT.

I have been working on a systematic review across the history of the literature documenting rates of comorbidities across this group of illnesses. And within ME/CFS, the ranges for these comorbidities include 12 to 70 percent of ME/CFS patients may have POTS, and 90 percent of ME/CFS patients have had reduced cerebral blood flow on a tilt. Twenty-two to 76 percent of ME/CFS patients may have fibromyalgia, though a meta-analysis revealed that number is 47 percent. Seven percent may have muscle activation syndrome or MCAS, though this is thought to be higher. And about half of long COVID patients have -- may have ME/CFS.

With that said, I'm going to do a deeper dive into the first couple of topics of our webinar today. There are significant rates of comorbidity between connective tissue disorders like hypermobile Ehlers-Danlos Syndrome, hEDS, and hypermobility spectrum disorder, HSD, and ME/CFS, POTS, and fibromyalgia. I'm working on a few projects on this.

And within the literature, in ME/CFS, 12 to 19 percent of ME/CFS patients may have hEDS. Fifty to 81 percent of ME/CFS patients may be hypermobile. In POTS, up to 31 percent of POTS may have hEDS. Up to 90 percent of hEDS and EDS patients, respectively, may have POTS, in the study of patients in EDS clinics. And in fibromyalgia, a meta-study found that the association prevalence, so how frequently they co-occur, between hEDS, HSD, and fibromyalgia, ranges between 68 and about 90 percent. So, that's a lot.

More research on connective tissue issues in long COVID is needed. But about 23 percent of long COVID patients in a large EHR study fit within a phenotype characterized in part by having higher prevalence of connective tissue disorders. COVID-19 is associated with a substantial risk for autoimmune and autoinflammatory connective tissue disorders in a large retrospective cohort study. Case studies document long COVID patients developing new joint hypermobility, post-COVID-19.

It's important to note that these rates of comorbidity are significantly higher than the general population prevalence. It's estimated that only 0.02 to 0.2 percent of the population may have

EDS, and 3.4 percent may have symptomatic joint hypermobility.

There are many outstanding key questions here, such as, are people of hypermobility at higher risk for these illnesses? Does their hypermobility become more severe or more symptomatic after developing these illnesses? Do some people with these illnesses develop essentially an infection or inflammation-associated connective tissue disorder? These are future research questions. And also, Dr. Ruhoy will be sharing some of her perspectives on this in the webinar.

I also want to mention that spinal disorders often related to connective tissue disorders are seen in many of these illnesses and that more research is needed on this topic. Upper cranial instability such as craniocervical instability or atlantoaxial instability, tethered cord syndrome, and Chiari malformation are some of these spinal conditions. These spinal conditions can result in neuropathy, brainstem, vascular and nerve compression, cord stretch injury, and cerebral hypoperfusion. Symptoms can greatly overlap with ME/CFS, including neurological, orthostatic, cognitive symptoms, fatigue, and pain.

Comorbidities for spinal conditions across these illnesses are significant. So, some statistics, an MRI study showed 80 percent of 205 ME/CFS patients have disc bulging or hernias in the cervical spine. Eighty-three percent have signs of intracranial hypertension. Sixty-four percent have obstructions in C1 to T2. Over half have had -- have spinal cord compressions. Over a quarter of ME/CFS patients have severe spinal problems, in an EHR study of ME/CFS clinics. Twenty-one percent of EDS patients have craniocervical instability, and 40 percent of EDS patients have tethered cord syndrome in an EHR study of EDS clinics.

Studies have found similar spinal conditions in the majority of fibromyalgia patients. Seventy-one percent of fibromyalgia patients have cervical cord compression in an MRI study. And 91 percent have short filum which can be indicative of cord stretch injury. Key questions include, what are the possible rules of infection, inflammation, and mast cell activation? We explore some of this in our webinar today.

Mast cell mediators like histamine and tryptase can directly damage collagen. That is something that Dr. Maitland will speak about today. Klinge et al., Dr. Klinge, found inflammatory cell invasion including mast cells in the filum of hEDS tethered cord patients. And their filum was half as elastic as non-hEDS tethered cord surgical patients. This increased their risk for cord stretch injury. Unfortunately, Dr. Klinge can't be here today to give her talk. But I encourage you to read her paper and findings regarding inflammation and tethered cord syndrome.

There is significant and emerging research on persistent infection in ME/CFS. Just one example is that HHV-6 microRNA, viral microRNA, was found in the brain and spinal cords of ME/CFS patients, including cervical, lumbar, and sacral nerve roots.

I'm now going to switch gears and discuss how we could integrate less studied pathologies into our studies of ME/CFS. I had the honor of serving on the Patient-Led Research Collaborative's Biomedical Research Panel in which we funded $5 million in grants to innovative research on long COVID and ME/CFS last year.

We required that all grant recipients screen for common comorbidities, including some of the less studied pathologies we discussed in this webinar, such as hypermobility, hEDS, and endometriosis, hoping that this would advance research on this topic. I'm listing here some of the validated surveys and questionnaires that we asked all grantees to incorporate into their studies.

And finally, I want to mention our Tal Research Group clinical study at MIT, whose PI is Mikki Tal. It is the largest clinical study in MIT history. And it is on infection-associated chronic illnesses, including acute and chronic Lyme disease, long COVID, and we're working very hard to add an ME/CFS cohort as well.

MAESTRO involves multiple different types of cutting-edge, non-invasive, neurocognitive, neurological, and autonomic testing, hypermobility and skin integrity testing, deep immune profiling, and several types of biological sample collections such as blood, urine, throat swab, saliva, optional fecal, vaginal, and menstrual effluence samples for multi-omics profiling.

I mentioned MAESTRO because while our focus is on neurological, neurocognitive, and immune testing, we are also incorporating assessments for many of the less studied pathologies that we discussed in our webinar. We do this not just in MAESTRO, but in our mouse research and other research as well.

So, one, we screen for comorbidities, for all common comorbidities and less studied pathologies discussed in this webinar, via medical history, validated survey, diagnostic criteria, and in-person assessments. Two, we screen for connective tissue disorders through Nevisense, for assessing skin integrity through the Beighton score, through hEDS assessment in person and by a questionnaire, and we incorporate an interest in connective tissue disorders post-infection in our mouse research.

For spinal conditions, we use medical history and symptom screen. But we go beyond this by adding a heel-toe walking as a possible indicator of tethered cord syndrome and changes in neck movements as a possible indicator of upper cervical instability. In our mouse lab, we are looking at infections of the spine and infection-associated chronic illness. We also hope to expand into neurosurgical tissue analysis and hoping to screen neurosurgical patients for these illnesses, including ME/CFS, and adding an MRI component to our research, where we screen for its

spinal conditions in addition to other pathologies.

For reproductive health, we screen via symptoms, medical history, and validated surveys. We collect vaginal swab, menstrual effluence samples optionally for multi-omics profiling. And I want to mention technologies such as the smart tampon from NextGen Jane that allows one to study the menstrual microbiome. We also conduct research on mouse models and endometriosis and reproductive tract pathologies in infection-associated chronic illnesses, which I will discuss in my next talk.

So, these are all just examples of how we might be able to add in examination of these less studied pathologies and these comorbidities into our ME/CFS research. So, I want to thank the Less Studied Pathology subgroup, Dr. Bateman, Dr. Hanson, Cort Johnson, Mike Hermus, Dr. Klimas, Vicky Whittemore, of course, and then also the Tal Research Group at MIT, led by Mikki Tal, and my colleagues there. Thank you so much. And we're looking forward to a great webinar today.

Vicky Whittemore: Thank you, Beth. There are a couple of questions for you. But I think what we'll do is move onto Dr. -- to the next talk who, I'll let you introduce the speaker, Beth. And then we'll address questions for both of you after her talk. All right. Back to you, Beth.

Beth Pollack: Thanks, Vicky. I also had a reference slide, but okay. I'm pleased to -- that Dr. Ilene Ruhoy is joining us today. Dr. Ilene Ruhoy, MD, PhD, is a board-certified neurologist and environmental toxicologist specializing in chronic and complex chronic illnesses. In addition to her private practice in Seattle, Washington, Dr. Ruhoy also serves as the medical director of the Chiari EDS Center at Mount Sinai South. She's the chair of the Neurology Working Group, working committee for the EDS Syndrome Society. She is talking to us today about connective tissue disorders and spinal conditions in ME/CFS.

Ilene Ruhoy: Hi. My name is Ilene Ruhoy. I am a neurologist, and I am the medical director of the Chiari EDS Center at Mount Sinai South Nassau. And I'm very honored to be here today to discuss connective tissue disorders, spinal conditions, and ME/CFS. And I thank the organizers, specifically Beth Pollack, for inviting me to be here today. I do not have any disclosures or conflicts. And this talk was not in any way generated with the use of artificial intelligence.

When we discuss heterogeneous disorders, we often are referring to diseases such as ME/CFS and even long COVID lately, as well as connective tissue disorders such as hypermobility spectrum disorders and hypermobility type EDS, as well as cranial cervical instability and the resultant cervical medullary syndrome.

The human body is composed of four major types of tissues: muscular, epithelial, neural, and connective tissue. And connective tissue is by far the most abundant and diffusely present. It is truly ubiquitous. It is everywhere in our body. It has structural and mechanical functions as it strengthens, it supports, it binds, it buffers, it protects. And based on where it is in our anatomy and what particular organ it is associated with, it has a different composition with regard to not only the fibril-forming collagen fibers, but also the extracellular matrix and its cellular components.

It supports the overall functioning of all body tissues. And it's important to note that the location of individual anatomical components and where they are in relation to other anatomical components of our body are genetically predetermined. And that's important. Because when anatomy doesn't sit where it's supposed to sit, even if it's off by 1 millimeter, then a lot of cellular signaling may be suboptimal. And for the most part, that doesn't necessarily pose a problem. But over time, and especially with a connective tissue disorder, it could potentially pose a problem, and symptoms can start, as well as disease.

And we have to think about the human body as this cohesive network of tissues. So, I don't just see patients as their central nervous system or their peripheral nervous system or their autonomic nervous system. I see it as a holistic cohesive network of tissues that communicate with one another and work with one another for overall support of physiology.

The most abundant fiber in the connective tissue is collagen protein, and it actually makes up about 30 percent of the body's protein. In fact, Malek and Koster, in 2021, studied EDS patients and hypermobility spectrum disorder patients and found that there was disorganized collagen bundles and abnormal cellular mechanisms of fibroblasts which make the collagen in the EDS patient population.

So, we often think of joint hypermobility when we're thinking of connective tissue disorders. But connective tissue, as I've said, is diffusely present in the body. And there are many, many

different types of connective tissue. In fact, blood is considered a specialized connective tissue. Its function is that it transports oxygen and nutrients and waste byproducts. And as we'll review, that is a major function that takes place within connected tissue as well.

The blood vessels are made of connected tissue. So, the outer layer of the blood vessels is called the tunica externa or the tunica adventitia. And that layer is a thick connected tissue with various amounts of elastic and collagenous fibers. And if we think about EDS patients and hypermobility patients, they are often difficult blood draws. And it's because their veins will roll away from the needle. And so, the question is, is it because of the adventitia layer being too thick that it doesn't want to accept or receive the needle? Or is it that the vessels which actually travel through fascia and connective tissue are not held tightly enough for the needle to access it easily?

Lymph is considered specialized connective tissue. It consists of cellular components, collagen fibers, and ground substance. And it supports the integrity of organ tissues because it actually removes waste byproducts, and it removes cellular debris.

Fascia is very much a connective tissue. It surrounds and holds every organ, blood vessel, bone, nerve fiber, and muscle in place, right? So, it's a sheath of tissue that encases or attaches and separates structures and organs of the body. There is both superficial and deep fascia. Fascia actually holds the different organs of our cavities in place. It actually protects our cavities, so the abdominal cavity, the pelvic cavity, the chest cavity. Fascia surrounds muscle bundles. It surrounds long nerves. So, it's very much a part of the overall body functioning and body system.

The bones are specialized connective tissue. It's made up of a calcified extracellular substance. It's got a matrix. It's made up of cellular components, including osteocytes, osteoblasts, and osteoclasts.

And the meninges are connective tissue. So, the dura is a very thick, fibrous membrane that's formed by dense regular connective tissue. It's much thicker than the arachnoid. It's a little stretched to cover the brain and the cord. And the collagen fibers have an indistinct spirality and are densely layered one above the other. And the composition and the architecture of the dura can change. And it actually can weaken, which is why patients with connective tissue disorders are very much at risk of dural leaks.

Cartilage is a connective tissue, of course. We think about it in terms of our joints. It's a flexible connective tissue. It's in our joints, but it's also in our ear, our nose, the discs of the spine, which we're going to talk a lot about. Hyaline is the most abundant type of cartilage, and it's very supportive and assists in movement. It protects our joints. And when it's compromised at any

level, our joints are very much at risk and are very vulnerable.

And tendons and ligaments are fibrous connective tissues. And a tendon attaches a muscle to a bone, but it also attaches muscles to other organ structures like the eyeball. And then a ligament attaches bone to bone. So, it very much assists in structural integrity and as well as movement.

So, connective tissue is made up of collagen. It's also made up of proteoglycans, glycoproteins, elastin, and extracellular matrix. And this is important because it's very complex. And that's the -- that's a very important point that I'd like to make with connective tissue. Collagen, there are at least 25 different types, in fact, a couple of more. Types I, II, and III are known to be the major components of the stroma. Type II is a major component in hyaline cartilage and vitreous.

Glycoproteins, for example, the fibronectin in the interstitium is a glycoprotein, the laminin in basement membranes and just about every organ we have holds a basement membrane. The elastin and the extracellular matrix, which is a very important component of connective tissue, it's made up of Collagen VI. And interestingly, there have been studies that have shown that Collagen VI plays an important role in the central nervous system and has links to neurological and neurodegenerative disorders. And when we think of diseases like ME/CFS, we think about a neurodegenerative disorder or at least a neurological disorder.

It also impacts several intracellular processes and pathways such as apoptosis and autophagy, cell stemness, differentiation, tumor progression, and macrophage polarization. And so, to consider these different collagen types might actually lead towards different ideas with regards to research in the ME/CFS world.

Cells of the connective tissue include fibroblasts which produce the collagen, the adipocytes, the chondrocytes, the osteocytes, the mast cells, the macrophages, the leukocytes, and the erythrocytes. And they all arise from a mesenchymal cell lineage, and they present all over the body, including the nervous system.

As I've already mentioned, the meninges and even the vessel walls, the cerebral vessel walls, but also the glial cells which make up 90 percent of the brain cells, and the rest are the nerve cells. And so, they provide sort of the matrix for those nerve cells, and they actually help to modulate and monitor input and output, facilitate communication.

The extracellular matrix which has non-structural collagen fibers, as well as proteoglycans, mucopolysaccharides, and glycoproteins. Now, the extracellular matrix is not just this static kind of matrix support. It's not a static structure. It actually has a lot of physiology going on, and it's very dynamic. It mediates exchange between the circulation and other tissues of the nutrients, of the oxygen, of the carbon dioxide, of the waste byproducts. It has metabolic -- metabolite

transfer, and it's also an immune -- a whole lot of immune activity.

So, it has basically, antigen-presenting cells that present these antigens, whether they're pathogens or proteins or a part of our own cellular membranes, to the immune cells of our adaptive immune system. And they also are where a lot of innate immunity takes place, so the mast cells and the macrophages, for example. And so, they are a true inflammatory and immune milieu, the extracellular matrix. And so, it's important to remember that the extracellular matrix is not just this static, non-dynamic, kind of structural and mechanical system. It really is where a lot of exchange and waste removal and repair takes place.

So, connective tissue disorders, they're heritable and acquired, right? So, over 400 different heritable connective tissue disorders have been identified, including Marfan syndrome and Loeys-Dietz and Stickler syndrome, but also many different skeletal dysplasias. But in the EDS world, there are 14 different clinical EDS subtypes that are recognized. And they comprise about over 19 different genes that have specific defects that have been identified. And these defects are in the architecture and metabolism of fibrillar collagens, of even their modifying enzymes, of extracellular matrix molecules, transcription factors, even components of the complement pathways, and more.

But the hypermobility type, the hypermobility spectrum disorder and the hypermobility type EDS, has been molecularly elusive. And so, is it monogenic? Is it polygenic? Are there variants that just predispose patients to hypermobility, or is it an acquired hypermobility syndrome? And I think that's an important point to make. So, is this an acquired type of EDS? And should we even be calling it EDS for that matter? But if it's acquired, so, then, what is it acquired from? And is it a pathogen? Is it viral? Is it bacterial? Is it an environmental contaminant or toxicant? Is it trauma-based?

So, that's an important point to make, that this might be an acquired form of a connective tissue disorder. In fact, there are several different connective tissue disorders that are considered acquired, such as autoimmune disorders which have long been thought to be collagen vascular diseases or connective tissue disorder diseases, including things like sarcoid and Sjögren's and scleroderma and systemic lupus erythematosus.

So, hypermobility type EDS companions or comorbidities, most of which we're not going to talk about because we have other speakers speaking about some of these, but tethered cord syndrome, cranial cervical instability, intracranial hypertension, intracranial hypotension, mast cell activation disorder, allergies, inflammatory disorders, POTS and dysautonomia, compromised immunity, such as autoimmunity. Other companions or comorbidities, so the median arcuate ligament syndrome -- so, these are all compression disorders -- superior mesenteric artery syndrome, nutcracker syndrome. Then there's hormonal dysfunction, so neuroendocrine axis

abnormalities and fluid imbalance, styloid hypertrophy, and sensory integration disorder.

So, interestingly, Taylor, in 2023, just recently here this past November, they looked at genetic risk factors for severe and fatigue dominant long COVID and commonalities with ME/CFS. And they found several different genes, but three of these genes are known to be associated with connective tissue.

So, the INSR gene which is an insulin receptor gene is found on cells -- on many cells, of course, but also on cells in the connective tissue. And the mutations of this particular gene can lead to insulin resistance, and insulin regulates collagen synthesis, cell proliferation, and extracellular matrix production. In fact, insulin resistance has been associated with connective tissue disorders, including scleroderma and Dupuytren's contracture.

They also found the GPC5 gene which is the glypican gene, which are cell surface heparan sulfate proteoglycans and have been implicated in skeletal dysplasias which is a form of a heritable connective tissue disorder. And then finally, they found that there are certain risk factors associated with the TNS1 gene, the tensin gene which is involved in cell movement and connective tissue development and is important for the structural integrity of blood vessels. And as I've mentioned, the outer layers of the blood vessels are made of connective tissue, as are the fascia and connective tissue of which those blood vessels travel through.

Now, joint hypermobility has been shown to be a risk factor for ME/CFS. Sixty percent of ME/CFS patients had joint hypermobility compared to 20 percent in controls that was found in the pediatric population by Rowe in 2017. That should be Rowe, R-O-W-E. This is autocorrected. It made it Rose. And then Eccles et.al, in 2021, found high rates of hypermobility in the adult ME/CFS population, and posited that with lack of identification of the hypermobility, we were risking these patients of having exacerbation of their disability, of their ME/CFS symptoms.

Also has been shown, however, not to be a risk factor specifically in the pediatric population by Vogel et al. in 2022. But hypermobility and craniocervical obstructions have been found to be overrepresented in ME/CFS patients by Bragee et al., in 2020. And these included cranial cervical instability, as well as internal jugular vein compression, leading to idiopathic intracranial hypertension.

So, connective tissue disorder symptoms that overlap with ME/CFS symptoms, I thought this was interesting. Because the more you look through the literature, the more you'll find that there's evidence of connective tissue involvement of some of the symptoms that ME/CFS patients suffer from, for example -- and this is certainly not a comprehensive or exhaustive list by any means, but myalgias.

So, Jones et al. looked at urinary and plasma organic and amino acids in chronic fatigue syndrome. And I'm always interested in urinary, organic, and plasma amino acids because they're very much a mitochondrial marker. And I very much believe that ME/CFS is somewhat housed in the mitochondrial dysfunction. And so, I'm always sort of interested in looking at different things like urinary organic acids and serum amino acids and acyl-carboxylic acid profiles.

But regardless, Jones, they did not find those previously reported abnormalities in urinary amino acids or organic acids in ME/CFS study. However, they did show evidence that patients with ME/CFS had low taurine and low histidine, as well as low alpha-aminobutyric acid that is associated with inflammatory diseases and for reduced intermuscular collagen with a lower threshold for muscle microinjury. So, when I think about the myalgias and the pain and the fatigue that patients with ME/CFS report, I found this study to be interesting.

And then even with regards to the fatigue, there have been studies that have shown that patients with connective tissue disorders have increased fatigue, pain, disability, and decreased general health, and then including endothelial dysfunction which has also been associated with long COVID. And we discussed how endothelial dysfunction with chronic inflammatory disorders such as inflammatory arthritis and connective tissue disease, may be associated with the poor integrity of the vessel walls. So, I found that these studies with regards to fatigue and ME/CFS and having -- with an association of connective tissue to be interesting.

And then gastroparesis, of course, is very much a symptom of ME/CFS, certainly slow motility is. And Grover et al., in 2011, showed increased connective tissue stroma. And then headaches, of course, there's a multitude of studies that have proven that hypermobility specifically and then other connective tissue components are very much associated with headaches and migraines. And then recently, Pollack et al., in 2023, showed that there's a high prevalence of gynecologic disorders in connective tissue disease.

So, intracranial hypertension, otherwise known as idiopathic intracranial hypertension, but I don't think it's idiopathic. There's the venous circulation of the brain. There's the dural sinuses and the internal jugular vein. And any kind of compression or occlusion acts like a tourniquet. And so, when there's a tourniquet effect, there's congestion, and that leads to brain swelling, and that leads to intracranial hypertension. And so, the treatment, of course, is stenting, shunting, or intermittent CSF withdrawal. And those all have their own various complications of which we won't go over. It's outside the scope of this talk, and I'm not a surgeon.

But it's important to note that when we have this kind of compression with a tourniquet type of compromise, then we have this backup. There's a congestion that backs up into the brain. And

then there's the swelling of the brain and resultant elevated intracranial pressure. And it's important to note that the fluid compartments of the brain all have exchange of fluid. In fact, that's how the glymphatic system works. And so, the arterial side, the venous side, but also the CSF compartments, is all in exchange of fluid. So, you back up one path or one road, and there's congestion of all.

So, the dural venous sinuses, so it's of the dura mater. So, it's between the endosteal layer and the meningeal layer. They're usually very closely united, except along certain lines where they separate to form these dural venous sinuses. And so, that's where fluid will back up, and there'll be congestion. And as I've said, it will extravasate into other fluid and volume compartments.

Intracranial hypotension, so the extracellular matrix alterations may contribute to loss of dural integrity causing weakness, tear, and meningeal diverticula. And so, as I've mentioned, the dura is very dense connective tissue and is very susceptible to weakening and to damage and to creating tears and out-pocking of the reticula.

The heterogeneity of connective tissue disease is a challenge for both diagnosis, risk stratification, and research. And so, it's sometimes really difficult to understand when and who and why, to further explore, you know, some of the symptoms that they're having. Because, you know, things like a headache can be very common amongst the population, and it's not just connective tissue disorders. So, it's sometimes the idea that a connective tissue component is involved can be very elusive. And I think we have to think about that a lot sooner, a lot -- and work towards at least ruling it out sooner rather than later.

So, a spontaneous CSF leak can, of course, cause intracranial hypotension. And that can come from meningeal diverticula, as I've mentioned, a dural tear, a dural venous fistula, Tarlov cysts which are these perineural sheath-like cysts that contain CSF fluid. And they can cause a lot of disabilities. But they are holding on to a lot of CSF fluids. So, the more -- the bigger that they get and the more CSF fluid that they hold, the lower the pressure is of the CSF compartment.

And then, of course, there's indeterminate. Sometimes we suspect a CSF leak. And we do a full comprehensive workup, and we just cannot find the leak. And so, this is a nice diagram showing the different kinds of cysts and diverticulum that can form, again, that take the CSF outside of the spinal canal and so then reduce the pressure within the CSF compartment of the central nervous system.

So, mast cells are of a sentinel location of the tissue environmental interfaces. And the dura contains mast cells, and they're very close to the nociceptive neurons of the dura. And they release mediators which initiate reciprocal communication between specific nociceptors on sensory nerve fibers.

And the dura mater mast cells express genes coding for mast cell proteases, specifically CMA1, and the inflammatory cytokine TNF-alpha, which can also be released under stress or from exposure, so infectious exposure or environmental exposure or just even a stressful exposure from physical trauma, emotional trauma. So, you know, it's important to recognize that the dura contains not only the sense-connected tissue, which is at risk of being degraded and compromised, but it's at risk of being degraded and compromised due to the concentration of mast cells that reside within the dura.

And, of course, the mast cells also sit amongst the perivascular spaces, specifically within the dura, which is not only filled with pain nerves, but also with vasculature. And so, on the brain side of the blood-brain barrier, they're strategically located to basically wreak havoc with that blood-brain barrier, including the CSF meningeal barrier.

And so, once those barriers are breached, then the nervous system is exposed to lots of different things that can enter that are not meant to enter, that the barriers are supposed to protect it from. And most of those things that can now enter create further neuroinflammation. And I think that's interesting. Because a neuroinflammatory response has been thought to be behind ME/CFS as well as long COVID. And so, the more that there is a neuroinflammatory response, the greater the symptoms of these chronic diseases will be.

So, connective tissue disorders and the spine. So, the spine is a series of joints, right? And so, when we think about connective tissue, we have to really focus in on the spine. So, we know that Chiari malformation Type I has been associated with connective tissue disorders, atlantoaxial instability which can lead to high cervical myelopathy, and myelopathy certainly has been associated with dysautonomia for sure, but also other kinds of symptoms such as paresthesia, weakness, vesiculations, you know. And myelopathy itself has been associated with connective tissue disorders.

Cranial cervical instability, which we're going to talk about, but it can affect the brainstem and the spinal cord through direct pressure from the cervical medullary syndrome, the vertebral artery involvement, and change in CSF flow because of obstruction. Basilar invagination which can also cause cervical medullary syndrome. Segmental instability and kyphosis which can also cause myelopathy because of the buckling of the ligamentum flavum. The tethered cord syndrome and Tarlov cyst syndrome, which we sort of mentioned already.

And the spine is really vulnerable, so much connective tissue, right? As I've said, it's a series of joints. And so, the discs are composed of the nucleus pulposus and the annulus fibrosus. The nucleus pulposus, which can be thought of as sort of like a ball, and it's meant to absorb compressive stress. While the annulus fibrosus is meant to protect the nucleus pulposus and is

made up of ligament and fibrocartilage. And the fibrocartilage actually comprises into vertebral discs in all vertebrate animals.

So, some connective tissues in the spine span the full length, right? The ligamenta flavum is between the laminae of all vertebrae and starts at C1 and ends in the sacrum. And these fibers found in the ligaments have a high degree of elasticity to prevent the spinal canal from buckling during extension.

The supraspinous ligament joins the tips of the spinous processes and spans from C7 to the sacrum. And the meninges that I've already mentioned, which the dura is dense connective tissue and the arachnoid not as dense but still connective tissue, protect neural tissue. And neural tissue is very vulnerable to compression. So, if it doesn't have that protection from the meningeal layers, it is going to compress. It will compress.

And this is a nice diagram of the different ligaments that hold the spine together. And it's important to recognize that, you know, at least for cranial cervical instability, the cranial cervical joint is actually held together by many different ligaments. And that is the wisdom of nature, basically, of evolution, of sort of trying to protect our spine and protect particular joints of our spine that house critical anatomy. And we're going to go through that.

So, Chiari I Malformation is a structural defect of the posterior fossa. It's characterized by a caudal descent into the spinal canal of the cerebellar tonsils. And it's considered a failure of occipital enchondrium leading to a small posterior cranial fossa. And it's either congenital or acquired. So, you can be born with a Chiari I Malformation, but it can also be acquired through connective tissue compromise basically. And that can be from either a tenderizing effect of muscle activation disorder, for example, or just chronic inflammation or exposure from an infection. It can also be due to trauma you can acquire a Chiari Malformation.

So, 10 to 80 percent, which is that -- you know, all across the literature you find different numbers. So, it's somewhere between 10 to 80 percent of the patients with Chiari I Malformation had EDS or another diagnosed connective tissue disorder. And then around 20 percent of the patients with Chiari Malformation I are considered complex Chiari, which means that there's also a syrinx that presents, but many have coexisting cranial cervical instability. Sixteen percent of the Chiari malformation patients have an occult tethered cord.

About 20 percent of the Chiari malformation patients actually develop scoliosis, and this figure will increase to 60 percent if a syrinx is present. And Milhorat, in 2007, evaluated Chiari Malformation I patients with hereditary disorders of connective tissue and found increased retro-odontoid pannus formation to be unique in Chiari malformation patients with hereditary connective tissue disorders when compared to Chiari malformation patients without hereditary

connective tissue disorders.

And so, this is a picture of a Chiari malformation. And you can see that the cerebellar tonsils are descending down into the spinal canal in the foramen magnum. And so, that foramen magnum, which is this hole in the skull, is considered a chokehold point. So, things sit in there and really don't have any room. So, the tonsils sit there. Sometimes the cerebellum sits there. The lower end of the brainstem sits there. So, everything is just sort of sitting in that foramen magnum. And there isn't enough room, which is why, historically, the answer was to decompress, which is to sort of do -- remove a piece of the occipital bone to allow for more room.

But when there's cranial cervical instability, we know that there is an increased risk of Chiari malformation, and that's due to the instability of the C0-C1-C2 complex which is the most mobile portion of the cervical spine. And so, the craniocervical junction, the CCJ, or it's sometimes referred to as the craniovertebral junction, the CVJ, consists of the occiput, the atlas and the axis, so C0-C1-C2, and also the clivus which supports the pontomedullary junction.

And so, we measure the clival-axial angle, which basically measures the potential brainstem deformity. And so, like I've already said, C0-C1-C2 is the most mobile portion of the cervical spine. It does 40 percent of our flexion-extension. It does 60 percent of rotation, and it does 10 percent of lateral bending.

And so, the CCJ contains cervicomedullary anatomy, specifically the lower end of the brainstem, more specifically the medulla oblongata. And it can cause what's called a medullary kink. Now, it results in what's referred to as the cervical medullary syndrome, which is not unique to CCI, right? So, if you had a tumor there or a raging infection there or some other reason for lots of inflammation, you could still have cervical medullary syndrome which can involve all the nuclei of the cranial nerves and the parasympathetic nervous system, but specifically, it affects the lower cranial nerves, which is IX, X, XI, and XII.

And this junction also includes the vertebral arteries, the basilar artery, the first and second cervical nerve roots, and CSF compartment flow. So, you have compression of blood flow into the brain, compression of blood flow out of the brain, compression of some of these lower cranial nerves, compression of CSF flow. So, sometimes there's obstruction of CSF flow where you can even have elevated intracranial pressure in the skull, but low pressure in the spinal canal or vice versa, or it could be the same throughout.

So, this is a picture of basically the brainstem and the upper cervical portion of the spinal cord. So, you can just sort of see where all the cranial nerves sit and where some of the important anatomy of the brainstem is. And also, it's important to note that the medulla oblongata houses nuclei. So, nuclei that speak to the hypothalamus, which is sort of the master gland of the

neuroendocrine axis, which is why we sometimes will see neuroendocrine axis abnormalities like hormonal dysfunction and fluid imbalance and so on.

It also houses nuclei for the autonomic nervous system. And so, it actually can compress and impair the parasympathetic nervous system because it's got nuclei that functions within that part of the autonomic nervous system, which is why many ME/CFS patients with CCI sit in a state of sympathetic activity, which is very draining to a lot of their glands of the neuroendocrine axis. So, that's an important point.

The clinical presentation of cervical medullary syndrome can be headache and neck pain, and as I've already mentioned, the lower brainstem and the lower cranial nerves, which are IX, X, XI, and XII. Nine, being the glossopharyngeal nerve, which provides motor and parasympathetic and sensory information to your mouth and throat, can cause dysphagia. Cranial nerve X, of course, is the vagus nerve, which is laryngeal and pharyngeal muscle dysfunction, as well as slow motility and other parasympathetic functions.

Cranial nerve XI is known as the accessory nerve which is a cranial nerve that supplies the sternocleidomastoid and trapezius muscles. And then the cranial nerve XII is the hypoglossal nerve which is mainly an efferent nerve for tongue musculature. And the nerve originates from the medulla and travels caudally and dorsally to the tongue.

And then spinal nerves C1 through S5 cause radicular pain, numbness, paresthesia, muscle weakness, vesiculations, loss of muscle mass, sensory ataxia. And cranial nerve -- I'm sorry -- cervical medullary syndrome -- autonomic dysregulation, can also be caused by cervical medullary syndrome, including dizziness, sleep apnea, motor weakness, sensory deficits, balance disturbance, vertigo, dysphagia.

And as I've already mentioned, the parasympathetic system, you know, modulates the cardiovascular system, the respiratory system, the digestive system. There's modulation of inflammation and immunity. There's effects on hormones and metabolism. So, I think it plays a very critical role in the symptomology of ME/CFS.

And when we think about compression of the medulla oblongata, we have to remember that, you know, it also contains the reticular formation, right, which sort of helps to modulate input and output, right? So, it gets input from the eyes, input from nerves of the ears, input from the external environment, from touch, pain, and temperature receptors. And it helps, of course, to modulate our sleep and consciousness, our central pattern generators, such as breathing and swallowing, cardiovascular control. It habituates all kinds of sensory input, so we know how to appropriately react and appropriately behave.

And so, this compression of the lower end of the brainstem, of the medulla oblongata is very much a part of what we think about with ME/CFS. And in fact, you know, we've had lots of patients with ME/CFS who have been really severely affected and are bedbound and have their cranial cervical instability surgically intervened upon with a craniocervical fusion, and they do well. They are no longer bed-bound.

So, cranial cervical instability, the ligaments are the major occiput-C1 stabilizing structures. Again, it's the most mobile portion of any other section of the spine. It's held together by many different ligaments such as the denticulate ligaments which have the most robust collagen tissues, the transverse ligament of the atlas which constrains the dens, and at alar ligaments from the dens they attach to the condyles.

And so, I think that it's important to recognize that one of the reasons why cranial cervical instability exists in connective tissue disorder is because of those ligaments. Because it's not held together well. And while there are so many ligaments there to try to protect that particular critical joint, when you have a connective tissue disorder, whether it's heritable or acquired, that joint is very much vulnerable and very much susceptible to becoming unstable.

And so, again, here are the ligaments and membranes of the cranial cervical junction. And it just sort of shows the different components of the different ligaments that hold this joint together and, again, try its very best to protect it.

So, Milhorat showed that there's unique mobility and morphometrics in patients with Chiari and EDS compared to those with only Chiari and not EDS or to healthy controls. And there are lots of different measurements that we do. We refer to them as morphometrics. And the clivus-axial angle which I already had mentioned, it shows a decrease in those with Chiari and EDS. There's also a decrease in the atlas-axis angle, as well as in the clivus-atlas angle. There's also a decrease in the basion-dens interval. And then there's an increase in the basion-atlas interval. And so, there are differences in the morphometrics of patients with EDS and Chiari.

And so, the normal anatomy of a clivus-axial angle. So, greater than 140 and 145 is considered normal. And then here is an abnormal clivus-axial angle which is linked to a stress deformity of the brainstem, so sort of a brainstem deformation because it gets stretched over the tip of the odontoid. And so, anything less than 135 is considered abnormal. Between 135 and 140, 145 is considered borderline, but anything less than 135 is considered pathologic.

So, the morphometrics in EDS and CCI, there are 20 different measurements that have been designed for the craniocervical junction. But most of them were designed for trauma or for static malformations, not for dynamic malformations. So, for CCI in the EDS population, we -- well, not we, but it's been brought down from 20 to about three different measurements, and that's the

Grabb's measurement, the clivo-axial angle measurement, and the BDI, which is a dynamic measurement.

And the three components of CCI -- of the cranial cervical junction instability, are horizontal, rotational, and vertical. In severe cases, all three components are compromised. And that's, you know, important because, you know, one of the things that we recognize when doing the invasive cervical traction is that, based on the severity of the cranial cervical instability, the invasive cervical traction may or may not be positive. In less severe cases, some are more effective than others.

The clivo-axial angle and the Grabb's measurements are more sensitive for horizontal instability. The basion-dens interval, the BDI, is a better parameter to gauge isolated or prevalent vertical instability due to cranial settling. And so, if there's significant cranial settling due to connective tissue compromise, we will better see the vertical instability with a BDI. I see here I'm running out of time. So, Grabb's quantifies the mass effect exerted by the tip of the odontoid, plus its surrounding ligament is complex on the adjacent brainstem. So, less than 6 millimeters is considered normal, greater than 8.5 is pathologic.

So, a special place, so everything seems to pass through here, the cranial cervical joint. Be there or be modulated over there. And the clinical presentations can be quite complex. And it can be confusing, and it can be hard to localize, frankly, and especially when patients are trying to just sort of describe what they're experiencing, what they're going through, what their days are like.

It can be very hard to really understand the concept of cranial cervical instability and the resultant cervical medullary syndrome and the resultant compression of the lower end of the brainstem, as well as the arterial and venous systems and the CSF flow. It can be very complex and confusing, for sure, but the more we understand, the more we'll be able to identify and acknowledge how all of this pathophysiological change is taking place.

So, basilar invagination, it's just another part of the cranial cervical complex, and it's commonly associated with Chiari and syrinx. And it also creates a similar set of symptoms, including headache and neck pain, aphasia, dysphagia, dizziness, neck weakness, paresthesia, bladder bowel dysfunction even. It's definitely made worse with neck flexion. And it's just, you know -- it's basically where there's a basilar invagination is an abnormality. The odontoid prolapses into already a very crowded space of the foramen magnum. And so, it creates sort of its own form of brainstem deformation.

And so, instability of the spine can also be seen with connective tissue disorders. So, there is scoliosis and kyphosis, which is an excessive deviation in the coronal and sagittal planes of the spine. And there's listhesis, where there's basically slippage of vertebral bodies over one another,

and it can affect the alignment of the discs as well.

And so, there's axial or rotational displacement of disc, causing spinal stenosis and further cervical myelopathy. And Rowe, in 2018, actually wrote how ACDF can seem to improve autonomic symptoms of ME/CFS patients just from the autonomic fibers that traverse the cord but also because of slowing of the cord and lack of blood flow of the cord. So, to replace the discs and correct the stenosis and correct the myelopathy, you can have a greater improvement in symptoms in ME/CFS patients.

Tethered cord syndrome. So, I'm going to skip this slide because I know Dr. Klinge is here to speak about this. And so, connective tissue is more than joint hypermobility alone. If it is, is it a secondary or even a primary component of the pathophysiology of ME/CFS? I mean, I suspect that it holds a greater level of importance and of significance in ME/CFS patients, as well as long COVID patients. I mean, the blood-brain barrier, you know, with the endothelial cells of tight junctions and pericytes of basement membranes and astrocytes end-feet ensheathe blood vessels.

I mean, loss of some of these barrier properties had been associated with neurological diseases and neurodegenerative disorders. And blood-brain barrier dysfunction can lead to ion dysregulation and altered signaling homeostasis. And when we think about how cellular signaling is so critical for the body's functioning, I think that this altered signaling based on even where organs and cells and tissues sit within our body and then within the ratio of each other, that the cellular signaling is suboptimal.

I mean, in neurology, there are a lot of migration disorders, for example, where cells don't migrate to where they're supposed to be simply because the signal dropped. And we consider that a migration disorder. And so, cellular signaling is of utmost importance. And as well as, you know, the immune function, right?

So, we know that -- for example, that neuronal degeneration and dysfunction can happen from chronic neuroinflammation. So, any kind of immune assault or infectious assault can cause an immune response, which causes neuroinflammation. And that, over time, can further degrade all the cellular mechanisms that go on within the central nervous system.

So, what we have stumbled upon, what we think we know, what we do not understand, what we have done, and what we are planning to do are important topics that we need to have further discussions on. We cannot see a pattern that lies in a direction towards which we are not looking. And we cannot see a pattern if our sample is too small. And we cannot unsee a pattern that we have seen over and over. And that, I think, is what I often try to explain.

We see this kind of pattern over and over again, with regards to things like cranial cervical

instability. You know, with cranial cervical instability, not only is there compression of the lower end of the brain stem and compression of all the arteries and veins and -- but, you know, the C1 then likes to move.

And then that C1 tubercle will sit on the internal jugular vein and cause compression. And that's one form of compression of outflow, but also styloid hypertrophy. The styloid process can sit on the internal jugular vein. And these are all associated with connective tissue disorders. And then there's further compression of the internal jugular vein and therefore, congestion and therefore, elevated intracranial pressure.

And so, you know, you see these over and over and over again. And you see improvement during invasive cervical traction. So, if you create space within that joint so there isn't as much compression and symptoms improve, I mean, that really says something. And it really tells us something. And I think it's something to follow up on to sort of understand more of the significance of these anatomical diagnoses. I don't think we pay enough attention to the anatomy and the importance of the anatomy and how things are supposed to be, where they're supposed to be, basically, and are supposed to be functioning and about how they're supposed to be functioning.

So, the definition of diagnostic criteria of Chiari I has been changing. That 5-millimeter rule that I was trained in during residency no longer holds true. Certainly, the diagnostic criteria of craniocervical instability in EDS patients has been evolving. Surgeons and surgeries are not equivalent.

And our center's criteria have been evolving. I mean, now, we actually -- we -- and for a while now, we've been -- we require the invasive cervical traction, as well as very often ICP bolt monitoring just to sort of prove that, you know, if CCI does exist -- which it very often does, but it doesn't mean it's contributing to the symptomology of the patient. So, we have to prove that it does in order to deem them surgical candidates. Because it has been shown to be a harbinger of a good outcome, of a good recovery from the fusion if indeed that's what's indicated.

And so, prophylactic surgery is not indicated. So, severely debilitating symptoms after failure of conservative management, then we consider CCI and its effects. We need positive imaging that supports it. We need morphometric that supports it. We need the ICT that supports it. Or if there's, for example, thought of Eagle syndrome, does the glossopharyngeal nerve block? Improve symptoms? Urgent versus elective? I mean, these are all things that we grapple with at the center.

And so, hypermobility type EDS, it's not a disease per se. In fact, it's an evolutionary advantage. In fact, they often say it's got protective qualities. But sometimes it backfires and produces

problems. And I think that's where the exposure component comes in. You know, pathogens and environmental exposures I think play a very important role. And I think there are probably genetic variants that make some susceptible to it and some not so susceptible.

It's got its own range of normal. I mean, to be born flexible is not a disease, right? It's why we have athletes and dancers. And again, it seems to be protective with regards to injury. But at some point, it can go awry. And then there's hypermobility and instability. So, being hypermobile is one thing, being -- having instability or being unstable is another.

So, next steps for research. We need to expand the knowledge and perspective of the role of connective tissue in ME/CFS. We need improved methods of evaluation in the clinic beyond the Beighton score. So, I think the Beighton score is great, and obviously, it's an easy thing to do within the clinic. And even patients can do it on their own at home. But we need better means of measuring connected tissue compromise and not just about joint type mobility.

We need improved diagnostic testing. So, imaging, labs. We need biomarkers. Lactate has been shown to induce collagen gene expression, for example. Hydroxyproline is a metabolite of connective tissue breakdown. We need biopsies. So, what tissues can we obtain and how do we -- what should we look for?

In fact, in our center, we're doing tissue studies, and we're looking at the histology of connective tissue. What are the abnormalities, and where is it? Is it in the structural integrity of the fibrillar collagen types? Or is it in the extracellular matrix? Is it of the cellular components or of the enzymes or of another part of the pathway of the development of the connective tissue?

And if it's due to -- if it's acquired and due to infection, then it's really a restricted region. And maybe that's why some patients have craniocervical instability but don't really have a history of hypermobility and are actually stiff. In fact, we have many patients that don't have a history of being flexible or hypermobile and actually will say, "No. I've been stiff my whole life. Everyone else has been more flexible than I." Because maybe it's been an exposure that has attacked a particular part of the anatomy. And so, it's a restricted region. And maybe those are where we should be collecting tissue samples from.

And so, exposures are -- you know, can be viruses and bacterium and environmental. And we should also look at germline versus mosaicism versus somatic mutations and variants. Because there likely is a genetic component for sure, but it's clearly not germline because we haven't really been able to identify what causes hypermobility type EDS. So, we need to look further within the genetics. And at our center, we're working on some mosaicism studies.

And then mitochondrial activity. I think that, you know, a lot of this is related to mitochondrial

dysfunction. It's likely secondary. But potentially, it's primary. And, you know, we've talked with some scientists with regards to different studies that we can do, not only with blood, but also with muscle biopsies and fibroblast studies.

And so, I think that there are different kinds of research options open to us that we should be collaborating on. And that's what we need to do. We need to collaborate further. And this is just sort of a nice diagram of the mesenchymal cell lineage. And that is my talk. Thank you so much. And I am ready for questions. And I'll be part of the panel later on today. Thank you so much.

Vicky Whittemore: Oh, thank you so much, Dr. Ruhoy. That was an amazing presentation. So, just for those of you who have joined, Dr. Klinge is not able to present this morning. So, we'll do about 10 minutes of questions right now. And then we'll break for the -- we'll take a break until 12:30 p.m. when we'll come back for Dr. Maitland's presentation. So, let me just go to the questions. So, I know some of them have been answered already. But we'll --

Ilene Ruhoy: I was answering during the talk.

Vicky Whittemore: -- hit on some of these because I think there's a lot of really excellent questions here. So, I think this is an interesting one. "Which collective or connective tissues are the trigger tender points found in fibromyalgia? And are they connected to oxygen and virus persistence?" So, what are the links there? And what are the -- what do we know about those triggers?

Ilene Ruhoy: That's a really excellent question. And we don't know a whole lot about those triggers. We know those trigger points are areas that seem to be of thinner fascial planes. So, they have much more access to nerve endings that lie underneath. But we -- so, those are what the trigger points seem to represent. But with regards to -- so, it's the fascia, basically. So, in terms of what type of connective tissue, it's mainly the fascia. But beyond that, we haven't done a whole lot of studies other than, we do know, you know, anywhere -- so, there's a -- there's several papers that are out. Some are actually, at this point, a decade old. But we've known for a long time that a majority of patients who have been diagnosed with fibromyalgia also have small fiber neuropathy.

And the papers -- you know, the range of -- percentage-wise is 40 percent to 60 percent of fibromyalgia patients have small fiber neuropathy, which I've always found interesting. Because it does show that there's something physiologic going on if the small fibers are involved. And they are pain receptors.

So, I think that that is what's behind the pain of fibromyalgia, is the small fiber neuropathy. And

it's probably a larger percentage than what's been reported. But it also, again, sort of sits in within the ME/CFS kind of septad patient, which small fiber neuropathy, of course, is one of those diagnoses that we very commonly make in this patient population. So, I think we need more studies to understand more behind the pain of fibromyalgia, which I think is, again, about the small fibers.

And I know it's often considered a myalgic pain, which, again, I showed a study that sort of suggested that there is low collagen within the muscles themselves which sort of -- a lot of my patients report sort of spongy kind of muscles or feelings of atrophy, though they don't really have any weakness on exam. And they don't really have atrophic muscles on muscle biopsies. So, it's sort of interesting that it's about collagen deposition. So, again, you know, not a whole lot of information. We definitely need more. So, those are good questions that I'm always trying to answer.

Vicky Whittemore: Great. Thank you. Another question. "Given that demographic incident rates are difficult to really determine for ME/CFS and related conditions, are there likely differences in connective tissue conditions and ME as a whole?" Or what are -- you know, I guess you could speak to the overlap there and what we know.

Ilene Ruhoy: Yeah. I think, again, one of the points that I tried to make is connective tissue is heterogeneous in itself, right? It's so diffusely represented in the body in different forms. And where it lies anatomically makes up how it -- what its composition is. And so, I do think that there are probably differences amongst the ME/CFS patient population with regards to what connective tissue is involved.

And I also try to make the point that not everyone -- although the majority are hypermobile, to be clear, but not everyone is. And so -- and I think that that's -- those are the patients -- yet they meet ME/CFS criteria. So, those are the patients that I think we also should focus on in understanding where their connected tissue is involved. But again, we don't -- you know, connected tissue is just sort of newly on the horizon with regards to ME/CFS. And so, I think that this is an area of study that really is begging for more research.

Vicky Whittemore: Yeah. I think the genetics findings that you presented are really interesting.

Ilene Ruhoy: Yeah. I agree.

Vicky Whittemore: Interesting to see if the -- that's replicated in the large DEVA study that's taking place in the U.K.

Ilene Ruhoy: Yes. Absolutely. Thank you for bringing that up. Yes.

Vicky Whittemore: Yeah. So, these two questions are somewhat related. So, "What role does polycystic ovarian syndrome play in insulin resistance connected to tissue -- connected to connective tissue disorders? And similarly, then what other gynecological disorders are you seeing connected to these disorders?"

Ilene Ruhoy: So, as a neurologist, I don't really see a -- I don't --

Vicky Whittemore: Sure. Fair enough.

Ilene Ruhoy: -- take care of a lot of those diagnoses. Obviously, a lot of my patients report that as, you know, as part of their medical history, certainly. And so, I find that interesting. And I -- but I don't really know enough to really say anything definitively or conclusively about those diagnoses specifically.

Vicky Whittemore: Okay. Beth, do you want to comment on that or -- I think we'll discuss that later. But I don't know, Beth, if you want to make a comment.

Beth Pollack: Yeah. We will be --

Ilene Ruhoy: I was going to say, Beth, maybe you should comment on that question.

Beth Pollack: Yeah. So, last year, I, along with a patient-led research collaborative, wrote the first review of reproductive health findings in long COVID, which we contextualized with findings in EDS and other associated illnesses. And reproductive health conditions are highly comorbid with EDS, especially a number of different pregnancy complications.

And I think there are important questions here in particular to -- regarding reproductive health issues around the role of sex hormones and how they may impact both reproductive health conditions and connective tissue laxity across the menstrual cycle. But I'll stop there. But it is -- it's a great question and a great topic to explore and research.

Vicky Whittemore: Right. Thank you. So, I think this is an interesting question. And you touched on this, Dr. Ruhoy. "Do you know if reduced blood-brain flow on tilt is caused by vascular response, parasympathetic response, mechanical compression, and/or other factors?"

Ilene Ruhoy: All of the above.

Vicky Whittemore: All of the -- I knew that was going to be your answer.

Ilene Ruhoy: I think it's -- yeah. I think it could -- you know, it depends upon the patient of course. But I think it could be a little bit of both. We do know that there is vasomotor dysregulation. Of course, there's a lot of dysautonomia in these patients. We think it's related to the cervical medullary syndrome because of the nuclei that are involved and that are compressed.

But we also think that there are other factors involved, again, just even based on the connective tissue component of the vessel walls. So, I think that it's really a little bit of everything. But again, based on the patient, it's probably -- you know, there's a probably more -- a more predominant etiology behind the symptom and the lack of cerebral blood flow. So, it's -- yeah. I think it's hard to sort of make these generalized statements because I do find it so individual. So, it -- yeah. I think we need to look further into that as well.

Vicky Whittemore: Yeah. I think there's questions about, how do you get to the cause or underlying cause of ME/CFS? And then this one question, "How do you decide on the ultimate cause of ME/CFS and how to focus your treatment when someone has cervical compression or other spinal abnormalities, yet there was a clear onset of illness after a viral infection?" Is that a coincidence?

Ilene Ruhoy: So, I -- yeah.

Vicky Whittemore: Or what's the correlation there?

Ilene Ruhoy: I mean, that's an excellent question. And we definitely grapple with that every day when we see patients.

Vicky Whittemore: Right.

Ilene Ruhoy: I think that the history is incredibly critical and incredibly important. So, just to get that kind of history that symptoms started after a viral infection is so important and needs to be focused on. Because we do know that post infectious or para infectious has an inflammatory response. And we know the inflammatory response definitely involves, obviously, a lot of immune cells, most predominantly the mast cells which have lots of different mediators.

Almost all of them are inflammatory. Some of them actually degrade connective tissue. So, there are proteases that are involved. There are mediators of the mast cells. And so, there definitely is a sequence of events that could potentially happen. I mean, obviously, I'm focusing on the connective tissue component of this. That was what my talk was about. That's obviously what I'm focused on. So, you know, when I hear this kind of story and I see any kind of spinal manifestation, I, sort of, you know, think about the immune response, the inflammatory

response, how it's degraded the connective tissue, and then where I go from there.

But with regards to starting any kind of diagnostic workup or even treatment, I really focus on what are the most, you know, predominant symptoms that is interfering in the patient's quality of life? What are they struggling with on a daily basis? And that's where I start. Because if I can get them feeling just even slightly better from a symptom that's just sort of overtaking their lives, then I can help them be a little bit more motivated to continue on with treatment that could potentially really move the needle for them. Which as you know, in the ME/CFS world, it's actually very hard to do. But we're finding that when we recognize these -- you know, whether it's CCI or a compression syndrome or some other spinal manifestation, there -- you know, we -- when we correct those kinds of anatomical concerns, we definitely see a significant improvement.

And we're actually, at Mount Sinai, we're going to be publishing a couple of papers this year that show pre- and postoperative changes. And a majority of patients, postoperatively, had significant improvement of the symptoms that they had had preoperatively. So, it does seem to support the idea that if there is an anatomical concern -- and again, it could be post-infectious or para infectious just from the inflammatory response. So, if there is an anatomical concern, then to correct that is not the -- is not a wrong recommendation to make. Right?

It's one of -- a good recommendation to make. I mean, obviously and rightly so, people don't want to have surgery. And again, you know, I understand it. And certainly, as a neurologist, you know, my end game is not that patients get fusions of their spine. But when it's going to make the difference in their health and in their quality of life, I fully support and advocate for it.

Vicky Whittemore: Great. Thank you. So, I think a related question -- we'll take this question and then we'll take a break. "For non-severe, non-bedbound cases with suspected neck compression or instability, is physical therapy aimed at the neck desirable? Or is this neck work risk stretching areas? Is it -- is there concern with physical therapy with someone who has suspected neck compression or instability?"

Ilene Ruhoy: I think physical therapy by an EDS-literate physical therapist is definitely warranted. You know, when someone understands craniocervical instability or even if there's, you know, any level of compression or any disc herniation or other -- you know, listhesis or other spinal manifestation. As long as the therapist is familiar with connective tissue and the spine for sure then I think it's actually, completely warranted and can -- I haven't seen it obviate the need for surgery if surgery is indicated. But if surgery is not indicated, physical therapy is a great therapeutic means of providing support. There is not great risk in the appropriate hands of a good therapist of weakening of the muscles. In fact, the paraspinal muscles will often spasm in relation to the instability of the spine. And so, sometimes, a good physical therapist can help

relieve some of that spasm which can be a source of pain and discomfort for patients. So, it can actually make them feel a little bit better.

So -- but again, it has to be with a therapist that completely understands an EDS body. Because an EDS body, you know, you move one part, another part can potentially move as well. And so, you want to keep things in alignment and keep things strong and flexible. And so, it has to be a very competent and literate physical therapist, of which there are many across the country, but you have to find one.

Vicky Whittemore: Right. Thank you.

Ilene Ruhoy: You are welcome.

Vicky Whittemore: So, thank you very much. There are so many questions we could get to. But I think we'll take a 10-minute break and come back at 12:30 for Dr. Maitland's presentation. So, thank you very much.

Ilene Ruhoy: Thank you.

Vicky Whittemore: Yeah. I'd like to welcome everyone back from the break. And I'll turn it back to you, Beth, to introduce Dr. Maitland. Thank you.

Beth Pollack: Thank you, Vicky. Up next is Dr. Anne Maitland, MD PhD, who's an expert in mast cell activation disorder. She's an associate -- assistant professor in the Department of Medicine, Allergy and Clinical Immunology at the Icahn School of Medicine at Mount Sinai. And she's the director of Allergy and Immunology Services at Metrodora Institute.

She's also a fellow of the American Academy of Allergy, Asthma, and Immunology, a member of the American Academy of Allergy, Asthma, and Immunology, and a chair of the Allergy Immunology Working Group of the National Medical Association. And she's talking us -- to us today about mast cell activation disorders in ME/CFS.

Anne Maitland: Oh, good morning. Good afternoon. I'm delighted for this invitation. So, thank you, Beth, and especially to be among such esteemed faculty. I feel like Rodney Dangerfield at the front row. But I'm also happy to share a cell population who probably feel that way as well, and that's mast cells, a cell population that was identified over a hundred years ago and really didn't get a job until 1989. And ever since then, it's been mis-profiled just as a bad actor that almost needs to be eliminated. But I have to tell you, mast cells are crucial for homeostasis, tissue defense, and tissue repair. And so, it would be important to understand how these cells may be misbehaving in the setting of someone who has ME/CFS.

So, these are my conflicts of interest which really should not pertain to most of this talk. These are going to be the objectives, which we were just talking about, the role of inflammation in ME/CFS and then the role of mast cell activation disease, which essentially means that there's a loss of tolerance, which can happen several different ways. And then I'm just going to share some clinical observations as well.

So, as has been exquisitely explained previously and the one thing I would like -- really like to highlight is that individuals have a tendency to suffer for months with a diverse set of symptoms before they get a better working diagnosis. And you can imagine somebody who has -- just has an uncontrolled asthma exacerbation for six months. And if you don't think remodeling in that organ system is not going to happen, leading to an allostatic negative change in someone's homeostasis. I think this is what is potentially happening between the conversation between the immune system, the nervous system, and within the connective tissue of various organ systems.

So, I love this comment by Dr. Bateman back in Medscape 2018. In a lot of ways, people think that ME/CFS -- a lot of practitioners and providers, healthcare professionals, are kind of overwhelmed by someone who presents with ME/CFS and often think that it's a very complex and not curable disease. But that's going to be the case if you think this is a one-trick pony kind of a syndrome instead of understanding that this is a question about communication and connectivity between the various organ systems, which you're trying to do on a moment-to-moment basis.

You're trying to take in oxygen, get rid of metabolites. You're breathing 18 to 20 times per minute. You swallow one to two times per minute, which increases when you are actively eating or drinking. Your skin is always exposed to the environment. And so, you have 75 square feet of territory that needs to be guarded to allow what you need for your biological machine to function and to get rid of metabolites. And there are going to be entities that are going to want to take advantage of that situation. So, backing up the border are components of the immune system to differentiate between danger and friendly entities.

So, possible causes of ME/CFS -- and this is a summary that came out of science maybe two

years ago, highlighted what has been already previously discussed regarding the role of infections and immune system changes. And there's a direct communication between infection exposures and immune system changes. And also internal changes, whether you're talking changes in the neuroendocrine system, whether this is a functional issue, as Dr. Ruhoy just discussed, which happens in individuals that have connective tissue issues.

And then structural issues at certain points of the body that are -- I almost would describe as watershed, whether or not it's a cervical spine, the occult tethered cord, or intracranial areas which may be more at risk for having compromised flow of the CSF, the blood, or the lymphatics that will lead to a change in the ability for that part of the body to receive what it needs to continue functioning and get rid of waste products that may be also detrimental to the function of that particular part of the body as well.

So, this kind of summarizes what has been put forth regarding possible causes of ME/CFS. And Dr. Ruhoy has exclusively talked about neurological alterations. And also, that kind of rolls into potential metabolic alterations, especially given the crosstalk between the neurological and the endocrine system. But I'm going to focus more on what has been talked about regarding immune dysregulation in the individuals that have ME/CFS, contributing to post-exertional fatigue, sleep disturbances, orthostatic intolerance, neurocognitive impairment, as well as fatigue.

So, this is -- I borrowed from the CDC, talking about what has been talked about regarding immune dysregulation in individuals that have ME/CFS. And there's been talk about chronic production of cytokines, of which there are numerous ones and different patterns that have been identified. You have low-functioning natural killer cells. And you also have differences in different T cell and, possibly, B cell populations.

And that's all well and good, but in many ways, we're really focusing on things that happen downstream after an initial insult. And again, in individuals that have ME/CFS, you're talking about six months of changes of which you're having a diminishing ability to take in what you need and get rid of products that you don't. And then you have essentially an allostatic negative change to your homeostasis which then can -- it's almost like a vicious cycle or circle that just does not come under control.

But what is sticking to me is like cytokines, natural killer cells, and lymphocytes are the help that gets called in. We're not talking about what happens at the initial site of injury, whether or not you're talking about an external or internal insult. So, we're starting really with the epithelial barriers, the connective tissue which has to sense whether or not this is a productive or non-productive environment for what they need to function and take in, again, nutrients, water, oxygen, and getting rid of the metabolites, carbon dioxide.

And so, once there's an injury, there are cells that are embedded in the tissue, including mast cells and dendritic cells. And what we find is they release chemicals that tell the rest of the body, there's a problem at this particular location and then will call in help, such as the natural killer cells, as well as call in help from the lymphocytes which typically are arranged at the lymphoid organs which are distant from the side of initial injury. So, we really need to focus on the epithelial lining, the connective tissue, and the first responders, of which the cytokines and natural killer cells and the lymphocytes are not participating. Again, they're the help that gets called in.

So, this is one model that I thought was helpful for trying to understand how this is kind of a vicious cycle that gets initiated by some response. This could be a motor vehicle accident. This could be the birth of a child. This could be some type of profound emotional loss. This could be a toxic exposure, since now we now live in an environment -- compared to two one or two generations ago -- where we spent 90 percent of our time indoors where we have increased exposure to indoor pollutants, including manufactured, such as formaldehyde, engineered hardwood, walls that may have asbestos in it, or biologics such as mold contamination.

And then depending on the resources that you have to detect that dangerous entity and then also have the resources to respond and contain it in an efficient fashion, you will be able to hopefully clear that response. But some individuals have ongoing exposure or have reasons for the immune system and the tissue injury not to subside.

And so, now you go from this prodromal period of time to early aspects of disease, where you have ongoing inflammatory issues both at the level of the epithelial linings, the connective tissue, and the innate immune compartment for which mast cells are key regulators. And then you have changes then that impact the adaptive immune system. And they all talk to each other. And that's what I think we really need to appreciate, is the crosstalk between all the components within certain organ systems and then the crosstalk between different organ systems that may lead to chronic neuroendocrine, autonomic immune, and circulatory dysregulation.

So, I want to kind of highlight how often mast cells are forgotten about. So, this is taken from a textbook on basic immunology, for which most of us as healthcare professionals receive maybe one or two lectures in a two-week course, first year of our graduate medical training. And often, when we talk about the innate immune system in the first line of defense, we talk about the epithelial lining and some of the components that it has. We talk about the white blood cells.

And again, we lean into macrophages, neutrophils, and natural killer cells. But again, those are the ones that get called into a site of injury. What's sitting at the site is mast cells and dendritic cells. And it is the chemical mediators that get released in distinct patterns that will influence what part of the immune response is going to be called in to counter the detected danger or

entity.

So, let's talk a little bit more about mast cells. Unlike neutrophils, natural killer cells, monocytes that get converted into macrophages, mast cells are born and -- are born in the bone marrow as well as the embryonal sac. And they are ubiquitous throughout the tissue. And they have a tendency to reside right at any border that is exposed to the external environment. And they also have a tendency to cluster near nerves, as well as blood vessels. Because then they can release chemicals that can help with the relay of help that call to say, "We're having a problem here. Please come in." And they also have a remarkably long life.

So, they're really educated by the surrounding microenvironment to say, "These are the needs we need to identify common, usual suspects that are dangerous to the homeostasis," and then also upregulate receptors as well as chemical mediators that are efficient at recognizing the danger containing it and calling in appropriate help if necessary. And this is, again, to emphasize the fact that mast cells are found in every part of the body.

But unlike how we describe even B cells and T cells, we still describe mast cells as whether or not you're located in the connective tissue, are you located in the mucosa, and whether or not you have another very potent enzyme called chymases within the granules that get released during degranulation events.

I love this article that was put forth by Dr. Theoharides who's been very active in mast cell disease, as well as in the ME/CFS world, has recently done quite a bit of work with Dr. Klimas as well. In that, we emphasize the fact -- and as Dr. Ruhoy just brought up -- mast cells are also intimately involved with our autonomic nervous system.

We find that mast cells are situated in the hypothalamus and pineal and pituitary glands. They have a tendency to cluster. And very interestingly -- and this might pertain to the question that was asked before regarding fibromyalgia and regionality -- mast cells have a tendency to cluster near acupuncture nerve sites. And depending on how you manipulate that acupuncture site, you can actually cause anesthesia or aggravate the pain syndrome.

So, just want to give you a visual of what mast cells are supposed to do. They really are kind of like the guards on the walls of any part of the body that's exposed to the external environment. So, you're talking about the linings of the respiratory tract, the gastrointestinal tract, the urogenital tract, as well as the skin. And they have to have receptors that have to recognize an array of potential injuries and dangers, including mechanical wounding, toxic exposures, whether naturally occurring such as venom injections, or manufactured, changes in physical parameters such as light, heat, water pressure. And also, a plethora of receptors recognize bacteria, viruses, and parasites.

And upon an injury signal, as well as receptor that recognizes a potential usual suspect, you have a distinct pattern of chemicals that are released to contain the danger that's been detected and then call an appropriate help. And so, to give you an example, if you are infected by a virus, the tendency would be to release chemicals that will call in lymphocytes. If this is bacteria, you're going to call in macrophages and neutrophils. And if it's a parasite, you are going to call in eosinophil. And the last thing you want to see is eosinophil in an environment that doesn't have something that requires releasing factors such as eosinophil-derived neurotoxin.

So, mast cells are, in many ways, ideal immune sentinels, given what they're equipped with regarding the detection of pathogens, as well as the ability to release an array of chemicals. And just to give you an idea of the potential chemicals that can be released not only through degranulation events, but also, for instance, if you go through the complement receptors, or you go through the immunoglobulin receptors. You will have different degranulation patterns compared to that, which is elicited by a G protein-coupled receptor called the MRG receptor.

If you go -- if the entity is recognized through the toll-like receptors, there's no degranulation event at all. You just have de novo synthesis of a range of mediators where you're either talking about prostaglandins, leukotrienes, tumor necrosis factor, chemokines that can call in lymphocytes, monocytes, or eosinophils, as well as factors that can alter the production of connective tissue, whether you're talking through the fibroblasts, macrophages, or monocytic platelets.

So, in many ways, these chemical mediators have the ability to do lots of things that are essential for our homeostatic existence. And that is, first of all, they release chemicals that have direct antimicrobial effects. They have the ability to recruit and activate various immune cells, as well as cells that support the connective tissue. They have the ability to alter blood flow. So, this is why you might see an increase in release of VEGF heparin or antiplatelet -- or a platelet-activating factor. And then also, they have the ability to help with tissue repair and remodeling after the danger has been quelled.

So, just to give you a kind of drill down the ability of mast cells to recognize of -- an array of triggers, you have both non-immunologic and immunologic activation. And what I mean is immunologic activation really focuses on specific antibodies or histocompatibility antigens that are on T cells or B cells to cause degranulation events such as you've developed an IgE to mold or peanut. And then upon cross-linking, you'll have degranulation events.

But then you also have the ability for direct activation through the toll-like receptors, through complement receptors. And I would like to kind of highlight one form of Ehlers-Danlos syndrome called periodontal Ehlers-Danlos, which interestingly enough, it is a mutation in the

complement system that is responsible for a global connective tissue issue, autonomic dysfunction, and increased susceptibility to mast cell activation events.

And then you also have chemicals that are released by surrounding cells such as estrogen and testosterone. You have compounds that are released, like endogenous opioids, as well as components that may be coming from the outside environment, from venom which has anesthetic properties, or botulism toxin that's released can actually go through that MRG receptor. And we see MRG to be very important for patients that have ME/CFS or Ehlers-Danlos syndrome or increased susceptibility to mast cell activation events, that this is a molecule that's very sensitive to certain anesthetic agents and also certain antibiotics such as vancomycin.

And to the right, this is just to emphasize the crosstalk between the autonomic nervous system and the mast cell compartments, where you have the ability for mast cells to recognize acetylcholine, ACTH, beta adrenergic receptors, which interestingly enough, in individuals that have vibration-induced urticaria, it is a mutation in the adrenergic receptor on mast cells that's responsible for causing individuals to have a physically induced susceptibility to mast cell degranulation events.

You have cortisol-releasing hormone, estrogen, NK-1, and NTR. All of these have the ability to cause release of different chemicals through the mast cell compartment, including histamine, endorphins, cytokines, bradykinin. Bradykinin is extremely important for how you constrict your blood vessels and as well as your smooth muscles of your airway. And so, you have the ability to modify any organ in the body, depending on how the mast cells are tickled and what chemical patterns are released.

And this is to highlight how there are different secretion patterns, either through degranulation or secretion from mast cells, depending on what the mast cells are seeing through which receptors. So, again, if you're going through the toll-like receptor, like for instance with SARS-CoV-2, which is recognized by several toll-like receptors, including 3 and 6, you don't have degranulation events. You're not having histamine or proteases releasing. But you are having different cytokines released, especially interleukin 6 which has been shown to be very important in ME/CFS, as well as long COVID.

If you're going through the antigen receptors such as the immunoglobulin G or complement receptors, again, you're having degranulation events with histamine being released. In the case of complement, you're also having tumor necrosis factor released. But you're having different patterns of cytokines and chemokines that are also released as well.

So, in many ways, mast cells possess sophisticated inflammation-processing in order to detect the surrounding microenvironment to see whether or not it is a positive or negative events -- a set

of events that are occurring. And depending on the alarm signals and the usual suspect that's detected, you're going to have different responses that -- in the form of different chemicals that are released through certain patterns.

So, what happens if mast cells start releasing these chemicals and you're not dealing with a parasite or a virus or such as -- or you don't -- you're unable to turn off the inflammation? So, you -- it's almost like a Goldilocks rule. You have to have just enough activity from the mast cells to contain the danger but not too much such that you're starting to have collateral damage in the area where you've experienced that insult and the possibility of that ongoing inflammation to go beyond the site of the initial exposure.

But depending on what the insult was, whether it's allergens, bacteria, viruses, parasites, mold, peptides, whether endogenous or externally introduced, certain medications or foods, you're going to have different patterns of chemicals that are released. And depending on where that exposure was, you can have systemic effects such as fatigue and weight loss. You can have upper or lower respiratory involvement, neurological involvement, musculoskeletal systems. We know that individuals that have clonal mast cell disease end up having increased susceptibility to osteopenia and osteoporosis. You have a myriad of gastrointestinal symptoms such as diarrhea, constipation, nausea, or vomiting.

Interestingly enough, one of the first papers talking about mast cell activation syndrome was a re-categorizing of individuals that had been suffering in an irritable bowel syndrome clinic at one of the Boston hospitals, where it was a revisit by both gastroenterologist, pathologist, and allergy immunology specialist that identified these individuals with the diagnosis of RBS, another disease description without defined pathogenesis or pathogenic mechanisms, where they found that these individuals actually suffered from mass activation syndrome. Changed the regimen that they had been taking for five years. And within weeks, these patients all reported partial or complete resolution of their symptoms.

You have skin manifestations, and of course, you have cardiovascular events. There's been plenty of data showing the crosstalk with individuals that have autonomic dysfunction such as postural orthostatic tachycardia syndrome. I think Dr. Rowe out of Johns Hopkins has mentioned how he's able to address individuals that have orthostatic intolerance in the setting of ME/CFS or long COVID, where you start not only tackling the autonomic dysregulation, but if you also help modify mast cell secretion patterns, you're able to help restore the vitality of the individuals and then start addressing their deconditioning that has been going on for such a long time with reintroducing appropriate physical therapy.

So, how do you diagnose mast cell activation syndrome which is a subset of mast activation disease? And the reason why I say that is, we're still lacking appropriate biological markers to

identify mast cell dysregulation, given that there's so many different patterns of chemical release from mast cells that can be triggered by an array of different insults.

But you start off with symptoms. And if you have dysregulation, if you have active symptoms in two or more organ systems, such as, you have airway congestion and gastrointestinal distress, you're having brain fog and pelvic -- bladder pain syndromes, any two or more of these, you should start evaluating the possibility that this person has mast cell dysregulation in the form of mast activation syndrome or mast cell activation disease.

You can get better with medications that target the mast cells, specifically if you're going through either the complement receptors or the immunoglobulin receptors with the use of histamine blockades, leukotriene antagonism, a monoclonal antibody that targets IgE, ketotifen, or hormones. But more importantly, it's important to have validated mast cell activation markers of this hypersensitivity event, including tryptase, metabolites of prostaglandin, or methyl histamine.

It -- but these are surrogate markers. If you really want to secure the diagnosis of mast cell activation syndrome or mast activation disease, you want to go into the tissue. And that's exactly what Dr. Klinge demonstrated with her evaluation of mast cell dysfunction in individuals that had occult tethered cord. So --

Vicky Whittemore: Dr. Maitland, I need you to wrap up in one minute, please.

Anne Maitland: No worries. So, we have various mast cell -- we have mast cell endotypes. And that's just to sort out, yes, your mast cells are overactive, but how can we help them -- it's important for a therapy to decide between mast cell activation disease caused by intrinsic defects or being externally driven. And this is just a cartoon to evaluate how you can do primary or secondary mast cell activation disease. And then depending on what is tickling your mast cells and what chemicals are released, you have different planned therapeutics that can be implemented.

And then also, it's really important to rule out that mast cells usually don't do this by themselves. And there may be other conditions that mimic mast cell activation syndrome, which is illustrated in this cartoon as well. I just wanted to highlight the fact that mast cell activation disease, as has been pointed out by other speakers, has been involved in lots of disease processes. So, it's really important to assess whether or not someone has mast cell dysregulation if they've been suffering with symptoms especially for months.

Again, leaning into this article from Dr. Theoharides, because there really haven't been many articles talking about mast cell activation disease and ME/CFS. These are the articles that were

put forth in this paper and highlighted the fact that there's only been three papers that have linked ME/CFS with mast cell dysfunction. So, when it comes to tackling ME/CFS, it's really one comorbidity at a time. More than likely, there's more than one comorbidity.

I wanted to share that a colleague and I published one paper showing the importance of identifying mast cell activation disease in individuals that had mast cell that had long COVID. And then there's this paper that Dr. Rowe and his colleagues showed that if -- he was able to get someone who developed COVID infection and had suffered long COVID. When he also addressed mast cell activation disease, he was able to get this person back to a better state of health.

So, in conclusion, mast cell activation disease -- there's a lot of circumstantial evidence that links MCAD -- MCAS with ME/CFS. But it's really important that we have more translational research to not only just talk about phenotypes, but the role of genetic products that are contributing to different phenotypes of ME/CFS. The epithelial -- the literature is definitely lacking on the crosstalk between the epithelial barrier mast cells and the autonomic nervous system.

I just want to show you, this is one genetic paper that identified numerous markers in the immune system, as well as metabolism and hormones have been shown to contribute to this insidious process in individuals of ME/CFS. And I just -- again, it's really important just to ask these questions. If you have a patient that has ME/CFS, do they have signs and symptoms of mast cell dysregulation? Do they get better with medications that target mast cells? What test results support that? And in what form?

And so, I'm going to round up right here. And this is how you can reach me. I'm now working at this wonderful institute in Salt Lake City called Metrodora, where we're trying to link various specialties under one roof to tackle individuals that have complex diseases.

Vicky Whittemore: Thank you very much, Dr. Maitland. There are some really excellent questions here that we'll come back to in the panel discussion later. But we need to move on to the next speaker. Thank you so much. That was great. I turn it back to you, Beth.

Beth Pollack: Hi. Yeah. It's my pleasure to introduce Julie Rehmeyer. Julie is an award-winning science and mathematics journalist and the author of "Through the Shadowlands: A Science Writer's Odyssey into an Illness Science Doesn't Understand." She has written for the *New York Times*, *Washington Post*, *Discover*, *Science News*, *Wired*, and many other publications. She's talking to us today about her lived experience with neurosurgical spinal conditions in ME/CFS.

Julie Rehmeyer: Thanks, Beth. I'm honored to be here. When I first heard of craniocervical instability in May of 2019, 20 years after I'd first begun developing ME/CFS symptoms, it was obvious that it did not apply to me. Neck pain was perhaps the 296th symptom on my list of concerns. I also didn't have Ehlers-Danlos syndrome which had generally been seen as one of the few conditions that could lead to CCI and, as Beth mentioned, is common among ME/CFS patients. My Beighton score was zero. I'd never been flexible. I'd never had subluxation in my life as far as I could tell.

My central problem quite clearly was MCAS, and particularly, hypersensitivity to mold, which patients commonly report as a trigger of ME/CFS, though the connection hasn't been researched much. Taking extreme measures to avoid mold had taken me to remission in 2013. But a subsequent series of exposures had gotten me very sick, again, with classic PEM, body-wide inflammation and pain, cognitive dysfunction, and all the rest. But with my relapse, avoiding mold hadn't been enough to restore my health. So, I was desperately looking for answers.

And one clue that seemed to fit with CCI was that some of my symptoms were clearly neurological. My legs got paralyzed and sometimes, much of my body. So, the next time this happened, I asked my husband, John, to give me traction. He pulled on my neck, and, voila, my legs started working again. So, suddenly, this implausible hypothesis seemed a lot more plausible.

So, my next step was to schedule an upright cervical MRI. I had to hold my head tilted back for several minutes while an image was taken. And I could feel my nervous system melt down over that time. I had walked into the building just fine, but I staggered out, pulling on my legs with my hands to get them to move. And after the MRI, anytime I leaned my head back even slightly, my entire body got paralyzed. Couldn't speak. I could barely breathe. And traction brought me back.

Loud sounds could paralyze me too, or too much talking, or a light tap on the head. I couldn't be left alone even for five minutes. We started joking that John wasn't just my husband, he was my necromancer. My MRI showed a problem. My clivo-axial angle was pathological, among other things. Essentially, the imaging showed that my top vertebra was pushing into my brainstem.

Putting together my symptoms with my clear imaging, Dr. Paolo Bolognese, a neurosurgeon expert in the field, diagnosed me with CCI. That December, Dr. Bolognese confirmed the diagnosis with invasive cervical traction. He put screws into my skull and used them to pull my skull up off my neck. I felt terrific. He loudly clanged on metal. No problem. I chattered away. No problem. Scans showed that my skull lifted away from my top vertebrae by 3 millimeters when it shouldn't have moved at all. Then he lowered the device, and out I went, unable to move, unable to speak, barely able to breathe. He lifted it, and I came back to life. We repeated

this over and over with the same results.

The next morning, I had surgery defuse my top two vertebrae to my skull, holding my skull in the correct position and giving my brainstem the room it needed. The next day, I was walking down the hospital corridor. Before we left, I was roaming the halls for an hour a day with no PEM. I hadn't been able to do that since my relapse. As I recovered from surgery and my MCAS and mold reactions lessened, nearly all my symptoms improved. Incredibly, surgery greatly improved my ME/CFS.

It's been a long road from there for me. As with many patients, I then needed tethered cord surgery. I re-tethered and had to have yet another surgery. And then I re-tethered again. I managed to deal with that last June. And I've gradually been improving since then. Each time I was tethered, along with extreme neurological problems, my reactivity increased and my ability to exert myself vanished. And each time I resolved it, nearly all my symptoms have improved.

It's not yet clear where my functionality will level out. But at this point, I may be 60 percent a healthy person which, for me, is pretty incredible. Among severe patients, it's clear that my neuro-structural problems are far from unique.

My doctor, David Kaufman, and my neurologist, Ileen Ruhoy, who we've heard from today, both say that bedbound ME/CFS patients should always be screened for CCI. A Facebook group to help ME/CFS patients navigate the diagnosis and treatment of neurostructural problems now has more than 6,000 members, with more joining every day. Many, many of them are finding problems along these lines, whether it's CCI or something else.

We don't have enough expert neurosurgeons to even evaluate all of these patients, much less to operate on them. And now we're seeing the same problems occurring in severe long COVID patients with ME/CFS, increasing the numbers even further.

But, of course, neurosurgery is a terrible solution, an absolute last resort. These surgeries are intense interventions that come with inherent dangers, have long recovery times, and carry long-term risks. We need to find a way to stop people from getting this sick, and we need non-invasive treatments for those who do. We have some clues to -- about how to do this because we know that strong immune reactions lead to the release of MMP-9 and other immune molecules that break down connective tissue.

My strong hypothesis is that my own spinal issues are a direct result of the chronic inflammation from ME/CFS and MCAS, perhaps combined with physical trauma from a bad childhood concussion. If we can stop the inflammation, we may be able to protect the spine. Thankfully, we now have a community of patients, doctors, and scientists researching this, including Beth

Pollack, who's chairing this webinar today, Mikki Tal, Ilene Ruhoy, Paolo Bolognese, David Kaufman, Amy Proal, Mike VanElzakker, Jeffrey Wood, Jennifer Bray, everyone in Alpha Tower, and more. Their work makes me look forward to the day when we'll be able to prevent spinal pathologies from occurring in ME/CFS in the first place and have nonsurgical solutions if they do. And then my blessed, life-saving surgeries will be viewed as crude or even barbaric.

So, the research priorities I would point to are these, how common are neurostructural problems in ME/CFS patients, whether mild, moderate, or severe? Are spinal conditions primarily a downstream effect of ME/CFS, or do they sometimes preexist and predispose patients to develop ME/CFS? How can we prevent ME/CFS patients from developing spinal problems? How can we treat spinal problems without surgery? What role do molds and mycotoxins play in the development of ME/CFS?

And then finally, we often ask about what the cause of ME/CFS is, but perhaps we're asking the wrong question. And could it be instead that these comorbid conditions reinforce one another, creating a kind of knot? In which case, there could be both many paths into ME/CFS and also, many paths out of it. Thanks.

Vicky Whittemore: Thanks very much, Julie. So, we were due to take a break. Are there -- let me see if there are any questions for Julie. Just a comment on your book. "It's incredibly powerful. Thank you." Someone asking about, "Where can you get an upright MRI?" You want to talk about that for a minute, Julie?

Julie Rehmeyer: Yeah. There's -- there -- you just will have to look in your particular area. There's a series of clinics, like Upright MRI of Colorado is where I got it done. They're in various states. So, Google is your friend here. And also, I would recommend, if you're looking into this, there's the ME/CFS+ Brain and Spine Facebook group which is a wealth of information. Really just hard to get through all of this without that information from fellow patients.

Vicky Whittemore: Just another question which I think is -- someone asks -- is -- "Were your symptoms, do you think, caused from tethered cord, mold, tethered cord, cervical instability, all of the above?"

Julie Rehmeyer: I think it's all of the above. Yeah. I absolutely think it was all of the above. You know, it's really hard to sort out like a kind of chicken and egg thing. And that was kind of the question that I was raising at the end. You know, maybe when we try to do so, we're asking the wrong question to begin with.

Vicky Whittemore: Yeah. Absolutely. I agree with that. What's the name of the Facebook

group that you mentioned?

Julie Rehmeyer: ME/CFS+ Brain and Spine.

Vicky Whittemore: Great. Thank you.

Julie Rehmeyer: And I also mentioned Alpha Tower which is closed but is an amazing kind of think tank of powerhouse patients who have been invaluable in pushing this forward.

Vicky Whittemore: Great. Okay. Thanks, Julie. I think we'll move on. Thanks so much.

Julie Rehmeyer: Thank you.

Vicky Whittemore: Okay. Back over to you, Beth. I think we'll skip the break because we're -- I don't want to get too far behind. Just move on to the next speaker. Thank you.

Beth Pollack: Yeah. And thank you, Julie. That was really meaningful. Dr. Laura Pace is up next. Dr. Laura Pace, MD, PhD, is a physician scientist specializing in neurogastroenterology, autonomic disorders, and genetics, with a focus on the diagnosis and care of people suffering from complex, multi-system disorders involving the neuroimmune axis.

She's part of the NIH Undiagnosed Diseases Network and is a board-certified -- is board certified in internal medicine, gastroenterology, and autonomic disorders, and has formalized clinical training in medical genetics and a PhD in neuroscience. She's talking to us today about neurogastroenterology in ME/CFS.

Laura Pace: Thank you, Beth. And thank you to the committee for the invitation to speak today. So, this is my brief outline. So, we're going to cover sort of, what do we know about gastrointestinal complications in ME/CFS? What do we need to know? And what are our research priorities?

So, let's start with what do we know? There's a lot to know. But what we do know is that greater than 90 percent of individuals with ME/CFS have gastrointestinal dysfunction. And some of the mechanisms that have been proposed include gastrointestinal dysbiosis and inflammation within the gastrointestinal system.

And so, if we look at the gastrointestinal system kind of up close, like within the small intestine, and that's what this picture depicts, is that the functions of the gastrointestinal tract at this point are really complicated. So, this is the largest interface of our nervous system and immune system with the outside world.

And what the gastrointestinal barrier has to balance is absorbing nutrients and water from our diet and keeping the outside environment, which includes our microbiota, outside. And so, in order to regulate what crosses the epithelial barrier within the gastrointestinal tract, they've evolved several ways to get things across that barrier. But obviously, things go wrong. And so, how do we measure currently gastrointestinal barrier permeability?

So, this would be sort of pathological barrier dysfunction. So, we can use small molecule probes. So, it's like you drink a sugar ratio -- a solution and measure the ratio of the sugars in the urine. You can use biomarkers from the blood. And then we can also use a technology called confocal laser endomicroscopy that measures it in real time and can -- is a really cool technology that I'll talk a little bit about later.

So, a study looking at mucosal barrier function in ME/CFS, they used the blood biomarkers to determine barrier permeability. And they found that a particular cow milk protein, zonulin, LPS, and then a binding protein of LPS were predictive for distinguishing ME/CFS and fibromyalgia from healthy controls but did not distinguish the two. So, it could not differentiate between fibromyalgia and ME/CFS. Interestingly, for both groups the more autonomic dysfunction is measured by COMPASS-31 correlated with increased intestinal barrier dysfunction. And why this is of concern is because if you have intestinal barrier dysfunction, you -- it's a trigger for systemic inflammation and sustained immune hyperactivation, which Dr. Maitland was alluding to in her talk.

And so then if we look at what the microbes are doing, there's been a few studies. And so, it's been confirmed that ME/CFS patients have substantial gut microbiota dysbiosis. They have changes in bacterial abundances, functions, levels of short-chain fatty acids. And this is

important because short-chain fatty acids like butyrate are the primary energy source for the epithelial cells that line our gastrointestinal tract. And so, if they don't have -- if those cells don't have enough energy, they can't maintain barrier function as well. And so, we've seen decreases in short-chain fatty acids.

We also have species interactions that deviate in ME/CFS. So, this study looked at the microbiota in ME/CFS using stool. And they found that there was reduced levels of Faecalibacterium prausnitzii and Eubacterium rectale in ME/CFS. And they thought that this may contribute to the deficiency that they noticed in butyrate levels because these organisms are known to be high butyrate producers. So, the low levels of F. prausnitzii abundance correlated with more severe fatigue symptoms in ME/CFS. But remember this is correlative not causative.

The next study I'm going to talk about, this is gastrointestinal microbiota in ME/CFS. And this is published as part of a collaboration through Jackson Labs. And so, they used multi-omics technology and identified a specific phenotypic gut microbial and metabolic biomarkers for ME/CFS patients. And they again showed reduced gut microbial diversity and increased levels of plasma sphingomyelin in ME/CFS patients. And that's a biomarker for disrupted intestinal permeability.

They interestingly broke the cohort down into short-term and long-term. So, your short-term patients are people who had had ME/CFS for less than four years. And the long term was people who had had ME/CFS for greater than 10 years. And what they found is that in the earlier course of the disease there's much more gut dysbiosis and decreased levels of butyrate compared to the longer-term patients where they didn't seem to have as much gut dysbiosis in their microbes. But they had more significant metabolic and clinical aberrations noted, and specifically more evidence of impaired gastrointestinal permeability because they had significantly elevated levels of plasma sphingomyelin.

So, really, we're already at the summary of sort of the work that's been done linking gastrointestinal dysfunction to ME/CFS. And so, what are the common themes? Well, it's common in ME/CFS. But you know what, that's not a surprise because it's common in many chronic illnesses. Dysmotility in ME/CFS wasn't specifically looked at. And so, there's really limited data. From my own clinic, I know that it's quite common. But we really do need more detailed neurogastrointestinal studies in ME/CFS specifically.

We know that gastrointestinal barrier function is impaired in ME/CFS. But again, this is no surprise because it's also impaired in many chronic illnesses. If we look specifically at what we found with the gastrointestinal microbiota, we have reduced diversity. But it's also common in many chronic illnesses. And interestingly, the studies that show this reduced diversity, they also have inconsistent results about which organisms are particularly up or down regulated.

And all these studies were done in stool. And we'll talk about why stool may not be the best proxy for the gastrointestinal tract as a whole. We do know that short-chain fatty acids play a role in gastrointestinal dysfunction. This has been proven in many other disease spaces as well, so again, not a common distinguishing feature of ME/CFS. Just like many other chronic illnesses, fecal microbiota transplants and probiotics have been proposed as therapies but haven't been specifically tested.

And so, what do we need to know? And I would say that we need to know an awful lot. And the reason I say that is that this system is incredibly complicated. And so, I'm just going to start with a big overview and sort of how I think about this problem. And so, the gastrointestinal tract is quite long. It is not homogeneous throughout, and that's not surprising because the functions of the gastrointestinal tract throughout its length vary.

So, you have the stomach which is primarily responsible for macromolecular breakdown. And then you have the small intestine which does more breakdown of the foods and medicines that we eat. But also, now you're having to have absorption across the epithelial barrier. You also have more interaction of the microbiota with the immune system at this area in the gastrointestinal tract. And that is intentional because we have a differently involved mucin subtype that is in the small intestine compared to the colon. And so, that's to allow absorption across the mucin and epithelial layer.

While the most abundance of the microbes are in the large intestine, they are not necessarily the ones that are having the greatest impact on the host. And so, most of the studies, not just in ME/CFS but across other disease spaces, are actually done on stool, so not even the colon. And so, we're missing an opportunity to really look at the microbial communities, their function in the area that has the greatest impact on the human host.

And so, the panels on the right side of the screen are really just showing sort of an up and close look at that epithelial layer with the overlying mucin. And you can see that the mucin's represented in green. And it's keeping the vast majority of microbes away from that epithelial layer. But if we have a breakdown in mucin production or any other dysfunction there, you have microbes that are truly interacting with the underlying epithelial layer and which is also meaning that they're interacting with the immune system and the nervous system that are also there.

And so then we're also going to zoom in a little bit more and look at the gastrointestinal tract in cross-section. And to make a point here, as gastroenterologists we are very adept at interrogating what the mucosal layer is doing during routine endoscopy. And in fact, we can take biopsies and send them to the pathologist or send them off for other studies. But the point that I want to make here is that the gastrointestinal tract is much more extensive than just the mucosal layer that we

can sample with those routine biopsies. And so, we're missing when we do that, looking at both the submucosal and myenteric plexus in detail. We're not looking at nerve fiber density. We're not looking at specific interactions between the nervous system and immune system and those types of things.

And so, we're missing an opportunity to truly interrogate the biology of the gastrointestinal tract when we are just taking surface level biopsies. And it really matters because we need to be looking at the immune system in more detail because the immune system of the gastrointestinal tract is functionally distinct from the systemic immune system and is not represented by sampling of blood. And so, we need more tissue-based analyses.

And so, this is why I like to think of ME/CFS as a neuroimmune axis disorder. And the reason is, is that, as everybody has pointed out, there are lots of -- we're going to call them comorbid conditions. But maybe they're actually not comorbid conditions and just a result of one underlying neuroimmune axis disorder. And, you know, if we look at the neuroimmune axis, you see that there's direct innervation and also signaling that occurs between the nervous system and the immune system and the endocrine system throughout our entire bodies. And so, we have to stop thinking about biology from a siloed definition that was created by medical specialties, and instead adapt a more systems biology approach. And so, we can't study one area without studying the others.

And so, if we look at why we need to really be thinking about these neuroimmune interactions, it's because well our knowledge has changed. So, earlier on we thought that communication within the nervous and immune systems, again, were distinct. And that only neurons use neurotransmitters. And that only immune cells use cytokines and chemokines. We now know that nervous immune and endocrine systems can all use neurotransmitters, cytokines, and prostaglandins to communicate which -- with each other using shared receptors. So, this is a major advancement and really changes the way we think about how these systems interact and how we have to study these systems differently.

And then if we add the microbiota in there because we know that they're directly interacting with the host, we have to consider that they are able to secrete substances into the bloodstream that can travel all the way to the CNS and have impacts on central nervous system functions. We also know that microbes can directly activate enteroendocrine cells. So, these are neuropods. These are newly discovered endocrine cells that directly synapses with the ENS and the ANS, which in turn is communicating with the CNS. So, again, you're having systemic level communication.

Microbes are also activating other types of enteroendocrine cells, which then release hormones that have both local and distant effects. Microbes can influence immune cell function and

therefore inflammation, which again is affecting the functions of the ENS, the ANS, and the central nervous system. And then a point I do want to highlight is that connective tissue is defining these interactions in all of our tissues. So, it goes beyond just joint hypermobility that we see in some of our patients. This is affecting all of our organs. And we don't have great ways to measure that.

So, if I sort of give a brief overview of how I think about this microbiota-neuroimmune axis and why we should be considering studying this in ME/CFS. It's because the neuroimmune axis plays critical roles in both coordinated communication between various body systems to maintain homeostasis and respond to environmental cues. And when it's dysregulated, you have symptoms everywhere. And this is what our patients are telling us.

The gastrointestinal tract is the largest interface of the nervous system and the immune system and the endocrine systems and also the largest intramucosal interface with the outside environment. The nervous, immune, and endocrine systems use a common language, so again, these neurotransmitters, cytokines, prostaglandins, and shared receptors. And they interact with each other. And so, we have to be considering that in our analysis of our data. And then microbes are also communicating with the host through this neuroimmune axis.

And so, when I think about a neuroimmune axis disorder, I think about sort of, like, four distinct features that every patient has. And this is whether they have a diagnosis of ME/CFS or they have a diagnosis of an autonomic disorder or other conditions that we commonly see. So, we see a lot of immune dysregulations. And for some people it's autoimmune, autoinflammatory, or innate disorders like mast cell disorders that Dr. Maitland spoke about or immunodeficiency or some combination thereof.

And the other thing that we appreciate is actually the immune dysregulation changes over time. And so, we need to understand the natural history of these changes. We also see neurologic dysregulation. And a lot of this is autonomic nervous system disorder. And since the autonomic nervous system is controlling the gastrointestinal tract, this obviously manifests with a lot of gastrointestinal dysfunctions as well.

And then we have connective tissue dysregulation. And both primary and secondary disorders of connective tissue are observed in this population. And while just like Julie mentioned, the Ehlers-Danlos syndromes are commonly the ones that we go to. They are a very small subset of the hundreds of connective tissue disorders that are defined. But there's also these secondary disorders of connective tissue that I think arise from dysregulation of the immune and neurologic systems.

And then the other piece that I think is really important is that there's clearly a genetic risk. So,

there's always a relevant family history of some sort of immune dysregulation and/or connective tissue component in families. But the scary thing that we're seeing is that it's increasing in severity across generations. And then the other thing that we really need to consider, and I think we could learn from, is the fact that all of these disorders that are neuroimmune axis disorders have a female predominance.

And so, why should we be concerned about this, not just for ME/CFS but to really move the needle on funding and understanding of these types of conditions? Is that 40 percent of people in the United States live with at least one chronic disease due to a dysregulation of the neuroimmune axis. Of that, you have 2.5 million people estimated to be living with ME/CFS. I think this is a gross underestimate. And unfortunately, is climbing every day due to our ongoing pandemic.

Likewise, we have 6 million people in the United States that have an autonomic nervous system disorder. And again, this is increasing every day because of the ongoing pandemic. We have 50 million people living with autoimmune disease. And most people don't just have one autoimmune disease. And so, it's something we have to consider about more broad dysregulation of the immune system to understand the biology of these conditions. And the other factor is women are disproportionately impacted by these conditions. And this is an obvious place of study. There are no cures for these conditions, and treatments remain extremely limited.

And if that's not enough to move the needle on why we need to do this and move with urgency, neuroimmune axis disorders account for 1 to $2 trillion of healthcare spending annually. So, not only are they devastating diseases, but they cost a lot of money. And so, our investment in research would be well won. So, the way I think about research priorities is more around the way I think about precision medicine and what we have to do for these complex conditions. And so, I think we need to really focus on deep clinical phenotyping.

And what I mean is even if someone has a label for what condition, we need to assess other things that are going on, including really in-depth assessments of neurologic function. And then if we want to talk about specifically in the gastrointestinal system, in-depth assessments of intestinal function, which includes motility and barrier function, microbiota analysis, and site-specific microbiota analysis. We need to move away from stool studies. We also need to do longitudinal sampling and spatial sampling.

We need to leverage deep immune profiling which includes tissue-based interrogation. Because again, the immune system of the gastrointestinal tract, like other tissues, is functionally distinct and we need to study it at its source to better understand it. And we can use tools like mass cytometry or single-cell profiling coupled with other technologies to really understand what the

immune system is up to in these areas. We need to look at complement pathways in more detail and cytokines.

And then we need to look at genetics. And I think this is something that every working group subsection has asked for. The technology has advanced extremely quickly, and the prices have come down, making this now something that should be part of routine clinical care. We need to start using whole genome sequencing to better understand these conditions.

We also need to consider using epigenetic profiling because we know so many people who end up in the ME/CFS space or broader neuroimmune axis disorder space end up here after a triggering event. So, it seems as though they have some genetic susceptibility and then -- in an environmental trigger that sets them off. And so, we could identify families that are high risk for the -- for developing these conditions and look at epigenetic profiles before and after disease onset. This would be very worthwhile to understand the biological drivers.

We also can use multi-omics technologies including transcriptomics, proteomics, metabolomics, single cell multi-omics, and CITE-Seq technologies to really understand not just which cells are where, but what are they actually doing. And this is really important for understanding the functional biology component.

We can use leading imaging technology. So, 3D tissue mapping, which I'll talk about in a couple of slides. And then, you know, the emerging data using 7-Tesla brain imaging in other disease spaces has been really illuminating. And we need to start looking at that in great detail in our patient population. We also can use functional studies like PET and spectroscopy to further understand some of the changes that may be occurring in this patient population.

So, now I'm going to switch gears a little bit and just talk about some of the specific technologies that exist within the domain of gastroenterology that we can use to understand some of these conditions and can be done as part of routine clinical care. So, this is confocal laser endomicroscopy. This is literally a confocal microscope that is placed down the working channel of an endoscope. And so, we can look in real time at a person's intestinal barrier function and we can determine whether it's intact or not.

We can also trigger this function with the application of either, excuse me, antigens or medications or additives or emulsifiers from foods or medications to see if those are directly disrupting intestinal barrier function in our patient population. This will help us understand what the immune system is reacting to. It'll also give us therapeutic interventions for patients such as removing certain foods or additives from their diet that may help them repair their intestinal barrier function, which again is only going to help downregulate that chronic immune activation that we see.

And the other things that we can do are utilize techniques where we can get a full-thickness biopsy of the intestinal wall. And this is really important because up until a few years ago, this required invasive surgery. And we know surgeries can be immune-activating events in this patient population and then set them off to massive clinical decline. And so, we want to avoid that at all costs. But now we can do this during routine endoscopy. And so, this allows us to get more than just the mucosal layer, which I can sample with routine biopsies. But now I can get intact microbial community structures.

So, for spatial biology, I can look at the submucosal and myenteric plexus. I can look at all the muscle layers, all the connective tissue distribution, and look at the interactions of the neurons and the immune cells as they are organized within the intact tissue. This is going to really move forward our understanding of tissue-based biology and these conditions.

And what we can couple this full thickness biopsy to is 3D tissue level mapping. And this is really important. Again, to look at the ultra-structural components of connective tissue, any changes that are occurring. We can look at ganglion density, nerve fiber density. We can look at blood vessel distribution density. We can look at lymphatics, all those types of things. And then we can also again look at in three dimensions, the interactions between the individual cells, particularly the neuroimmune and endocrine cells, and really understand, what are the differences compared to healthy controls?

It's also going to allow us to select therapeutic targets. There's a lot I think we can do with advancing our care for patients and for future studies regarding medical interventions. I'm just going to briefly touch on medications targeted at mast cells because mast cells really are causing a lot of havoc in neuroimmune axis disorders, including ME/CFS as Dr. Maitland emphasized. And so, typically we're using drugs that either inhibit mast cell mediators or kind of stabilize the mast cell as it is to reduce its release of mediators.

There are drugs in development that are actually much more powerful than the drugs we're currently using. And these are targeting Siglec-6 receptors. And so, these are receptors, when activated, can shut off all these signaling receptors in mast cells. So, you can simultaneously kind of hit all the possible ways that a mast cell can be activated. And they're also interestingly leading to mast cell depletion through an antibody-dependent cellular phagocytic mechanism. And so, we can selectively reduced -- reduce mast cells as well. These need to be studied.

The other future therapies that I think should be studied are, again, immune system modulators. There's a lot of monoclonal antibody therapies already out there and FDA approved for other indications that I think could be leveraged against ME/CFS. There's a number of small molecule drugs as well. And really repurposing these immunotherapies, I think, are going to be really,

really helpful. Because, again, it does seem as though this immune dysregulation is one of the major drivers of many of these conditions, including ME/CFS. And if we could get to the bottom of that, and as Julie mentioned, intervene earlier, we likely can prevent the clinical declines seen over the natural history of these conditions and prevent many of the severe cases. And as she said, barbaric interventions.

We also can reprogram the immune system, T cell reprogramming, CAR-T therapy. There's also CAR-NK therapy now. There's a lot that we can do. And we need to be leveraging these technologies against this devastating illness. There's also the work of Kevin Tracey using bioelectronic medicine and many others particularly around vagus nerve stimulation, transcranial or trans-spinal magnetic stimulation as well that's reprogramming the nervous system in a very interesting way and having impacts on immune system regulation.

And so, just to summarize, I really think we need to approach ME/CFS from more of a systems biology perspective and consider these conditions more as a neuroimmune axis disorder. And I really agree with Julie on this. I think the inciting event may not be relevant. I think it's just an event that activates the immune system. And if we thought about these conditions as sort of a post-immune activating condition, we might get a little bit further with understanding the biological drivers of these conditions and places for intervention.

I also think we need to compare across other disorders of the neuroimmune axis because I think they're related. I don't think these are separate. We think of many of these conditions like POTS, MCAS, EDS, Sjogren's syndrome, et cetera, as comorbid conditions. But maybe they're really just all part of this neuroimmune axis dysregulation that we see to some degree in neuroimmune axis disorders. And I think kind of taking a comparative biology approach would be really helpful. So, instead of looking across species, we're actually looking across these clinically-named illnesses that have very poor biomarkers and looking at them as a larger group.

I think we need high-quality data from deeply-phenotyped patients. And I believe that deep clinical phenotyping can and absolutely should occur as part of routine clinical care. And the reason I say this is this is a devastating illness, and we have to move with urgency for our patients. We need to leverage leading therapeutics for our patients as well. Drug repurposing, they're doing it in other spaces. We are behind on this. And we really should move this to the forefront of the way of our thinking for our patients.

And then we need to leverage leading technologies to really solve ME/CFS. And again, I'm just going to come back to sort of like GI-specific things beyond everything else that I mentioned. But spatial biology community and single-cell physiology studies of the microbiota are really important. And we have to expand these studies to stop focusing only on stool and really look at the region of the gastrointestinal tract because these are distinct communities with distinct

functions. And I think that that will really expand our understanding of this microbiota-neuroimmune axis in ME/CFS and other neuroimmune axis disorders. And the final few slides are really just references that I put in for everyone to have. And thank you again for your attention and the invitation to speak.

Vicky Whittemore: Thank you so much, Laura. That was excellent. So, I heard a new term, new for me anyway, neuropods. Can you say a little bit more about what neuropods are?

Laura Pace: Yeah. So, they're a newly-discovered enteroendocrine cell that's directly synapsing with the ENS and the ANS within the gastrointestinal tract. And so, they do have basically like a nerve terminal and that's how they're communicating.

Vicky Whittemore: Interesting. So, some questions that came in. Can permeability to diet-sensitive metabolic changes attribute to gut barrier function? Or maybe is it more attributed to metabolic control by hormones? In other words, what might be a good metabolic biomarker for gut permeability? How do you look at gut permeability?

Laura Pace: Well, there's a number of -- so, I quickly reviewed it. But there's a number of biomarkers that we can use that just show that we're getting kind of more microbes across that barrier than we should be. And LPS is one and then the proteins that bind LPS. The metabolic disturbances, I don't think we have good biomarkers for that, but we definitely see metabolic disturbances in our patients.

I do think in general, for everyone, but definitely in the ME/CFS population, we need to be focused on diets that reduce processed foods and are increased in dietary fiber because that's going to support a healthy microbiota and, again, the short-chain fatty acid production, which again is going to help with intestinal barrier function. And so, those are things that we can do. But really, there's so much more data that -- that's needed before I can definitively answer that question.

Vicky Whittemore: Yeah. And I really agree and appreciate with your comments about both not siloing our studies, but really looking across systems and also looking specifically at different areas of the gut. How do you propose doing that? How are those studies done?

Laura Pace: Well, I mean, like, I'm a gastroenterologist. And so, I do endoscopy all the time. And these patients are getting endoscopies done for their symptoms, right?

And so, we can collect those samples during routine endoscopy. And we really need to be doing that. I have data that's unpublished that shows that stool is not a good proxy for anywhere in the gastrointestinal tract, not even the colon. And so, we're probably missing a lot of the biomarkers

that we should be looking at. It doesn't mean we may not be able to later go on and correlate that with what we detect in stool. But I really think it matters where we're looking. And the samples are easy to get for any gastroenterologist.

Vicky Whittemore: And just one final question, your thoughts about fecal transplants. Do -- I know that there is a clinical trial that's planned. I'm not sure it has actually started enrolling patients in the U.K. yet. But what's your thought about fecal transplant for ME/CFS?

Laura Pace: Yeah. So, it's an interesting question. So, I would rather talk about it as, like, a microbiota transplant.

Vicky Whittemore: I agree. Yeah.

Laura Pace: Because, again, we can live without our colons. Stool is probably not a good thing to be replacing. We should actually be doing sort of constructed communities. And it's probably much more important that we have a healthy microbiota within our small intestine because that is the -- those communities are the ones that are helping us break down our foods. They're the ones that are interacting with our immune system more directly, those types of things. Whereas, like, the colon one, I just -- I don't think we should be using fecal microbiota transplant. And if we do transplant, I think it should be focused more on the small intestine.

Vicky Whittemore: Thank you. Okay. Thank you very much. We're going to move on. Thank you very much, Laura.

Laura Pace: Thank you.

Vicky Whittemore: Excellent. So, the next speaker is Beth Pollack who is going to talk to us today and give an overview of prevalence and key topics in female reproductive health conditions. Beth?

Beth Pollack: Thank you, Vicky. As Vicky mentioned, I am a research scientist at MIT. I lead research on the overlaps and shared pathophysiology across infection-associated chronic illnesses in the Tal Research Group, which is led by Mikki Tal. In my talk I'm going to give an overview of female reproductive health findings in ME/CFS. I will discuss these in relation to findings in Long COVID, POTS, and EDS. And I'll discuss emerging endometriosis research and endometriosis across these illnesses. And finally, I'll provide an overview of the reproductive health section of our webinar. Let's begin. I have no disclosures.

Great. I worked with my colleagues at the patient-led research collaborative, including Emelia von Saltza who's speaking in the webinar, to write the first review of the reproductive health impacts of Long COVID. We contextualized these findings by drawing on research evidence of female reproductive health in illnesses that are often comorbid or may be associated with Long COVID such as ME/CFS, POTS, and connective tissue disorders like Ehlers-Danlos syndrome, or ES. All of these illnesses disproportionately impact female patients, which make up 63 to 83 percent of the patient population across illnesses.

Pre-menopausal women have a higher risk for Long COVID and possibly for ME/CFS suggesting that sex hormones and sex differences in immune responses to infection may play a role. To summarize our finding, these illnesses individually have increased rates of infertility, endometriosis, ovarian cysts, uterine fibroids and bleeding, lacking menses, irregular and inconsistent menstruation, intermenstrual bleeding, menstrual cramps, vulvodynia, painful intercourse, early menopause, pelvic congestion syndrome, gynecological surgeries, and adverse pregnancy complications such as preeclampsia, maternal mortality, and premature birth.

Half to two thirds of female ME/CFS patients report increased illness symptoms before menstruation. This indicates that it may be important for us as a field to consider the menstrual cycle while conducting patient symptom surveys. Women with ME/CFS compared to controls disproportionately report irregular menstrual cycles, amenorrhea, dysmenorrhea, excessive menstrual bleeding, and bleeding between periods. Reproductive health conditions are common yet understudied in ME/CFS. Patients report increased rates of polycystic ovarian syndrome, ovarian cyst, pelvic pain, gynecological surgery, and endometriosis compared to controls.

A longitudinal case control study revealed that early onset menopause is a risk factor for ME/CFS. And menopause exacerbated symptoms in 38 percent of perimenopausal and postmenopausal women with ME/CFS. A study found that women who had been pregnant in previous years were 31 times more likely to develop ME/CFS. Pregnancy is reported as a trigger of both ME/CFS and POTS accounting for 3 to 10 percent of cases. And results are mixed on this. But pregnancy exacerbated symptoms in large subsets of ME/CFS and POTS patients. Dr. Boneva will be going over this in more detail in her talk later in the webinar.

ME/CFS, POTS, and Long COVID shared many reproductive health conditions, symptoms, and menstrual irregularities. I'll review some of those findings here. Few studies have investigated the impact of Long COVID on female reproductive health. That said, we found emerging evidence suggests similar to ME/CFS, Long COVID can disrupt the menstrual cycle, gonadal function, ovarian sufficiency, menopause, and fertility.

Similar to ME/CFS and POTS, studies have found increased rates of dysfunctional uterine bleeding, secondary amenorrhea, uterine fibroids, endometriosis, ovarian cyst, and pelvic congestive syndrome. In terms of menstruation, menstrual cycle irregularities are common in Long COVID patients including changes to the length of their cycle, duration, and intensity of menses. Like ME/CFS, studies show that between one third and 62 percent of premenopausal Long COVID patients commonly experience an exacerbation of Long COVID symptoms in the days before menses, as well as worsening of premenstrual symptoms. This is a common trend across illnesses.

In a patient survey of nearly 1,800 menstruating Long COVID patients, 34 percent report menstrual issues. And within that, 26 percent had normally irregular cycles. And 20 percent had heavy periods. In POTS, menstrual cycle hormones have been found to affect hemodynamics and menstruation is associated with an increase in dizziness. Similar to ME/CFS, premature menopause has been found to possibly be more prevalent among Long COVID patients in their 40s than in the general population. Few studies have investigated Long COVID in pregnant people.

A small, control-matched prospective cohort study in Brazil followed patients after testing positive for COVID-19. 76 percent of them developed Long COVID in this 84-person study. This is possibly reminiscent of research that has found pregnancy as a risk factor for ME/CFS. In EDS, if you look at the pregnancy column, it is associated with an increased risk for host of pregnancy complications. Limitations of many of these studies include a lack of healthy control or comparison groups, not screening participants for comorbid illnesses, and a lack of consideration in the study design for factors that may impact menstrual cycles such as vaccination. So, again, I stress the need for more research and reproductive health in infection-associated illnesses.

Here I'll discuss endometriosis. In particular, research suggests that between 20 and 36 percent of female ME/CFS patients report endometriosis and 20 percent of POTS patients. This is two and three -- two to three and a half times the general population prevalence. Critically, the average time to diagnosis from symptoms onset is 7 to 10 years. In regard to Long COVID, a study of non-hospitalized Long COVID patient -- patients -- COVID patients, sorry, found that patients with endometriosis may have an increased risk of developing Long COVID. But more research is needed to understand contributing factors.

People with polycystic ovarian syndrome were found to have a 28 percent increased risk for COVID infection after adjusting for other risk factors. Similarly, research suggests that ME/CFS and endometriosis may share some overlapping immune dysfunctions, which we discuss in our review. But I'm not going to go into detail because I think Emelia von Saltza will go into this later in the webinar.

So, this is a high-level overview. So, I'm not going to go too deeply into discussion of possible mechanisms as I just mentioned. But I do want to mention, a very interesting area of emerging research is on whether infection and possibly immune responses to infection might contribute to the development of endometriosis. Last year, a study found that fusobacterium in endometrial tissue was present in 64 percent of 79 women in -- with endometriosis and just 7 percent of healthy controls. In mouse models of endometriosis, mice inoculated with fusobacteria had increased in larger endometrial lesions. Antibiotic treatment reduced fusobacteria and the number of -- in size of endometrial lesions in mice.

In the Tal Research Group at MIT led by Mikki Tal, we are studying mouse models of infection-associated reproductive tract pathologies. I'm very excited to share with you some striking preliminary findings. If you look at the right of my slide, the mouse on the left is an uninfected mouse. It has normal uterus, ovaries, and reproductive tract. The mouse on the right was infected with a pathogen. And you can see that it developed severe reproductive tract pathologies including enlarged, inflamed, and infected uterus, ovaries, and reproductive tract. Stay tuned for more research from us on this.

There are many outstanding questions about the roles, mechanisms, and impacts of reproductive health conditions in ME/CFS as well as in Long COVID, POTS, and EDS. We highlighted some of that in our review. They include examining the roles of sex hormones and of mast cell activation within the menstrual cycle, related fluctuations of illness symptoms. Additionally, how might hormone fluctuations in the menstrual cycle impact connective tissue laxity, and how might this contribute to illness symptoms?

Important areas of future research include examining the menstrual and reproductive tract microbiomes in ME/CFS, which I mentioned a bit in my first talk. And exploring possible pathogen persistence in the reproductive tract, which we are doing in our clinical study at MIT. It will be important to develop less invasive, diagnostic approaches for endometriosis and reproductive tract pathologies and to better understand their roles in these illnesses.

So, I'm going to finish my short talk on reproductive health in ME/CFS by overviewing the health -- the reproductive health section of our webinar. So, I just kicked it off with an overview of female reproductive health and endometriosis and contextualizing this with findings in Long

COVID, POTS, and EDS. Next, Dr. Natalie Thomas will be discussing neuroendocrine dysfunction and the role of sex hormones in ME/CFS. After that, Emelia von Saltza will give a lived experience talk on endometriosis and ME/CFS. And finally, Dr. Roumiana Boneva in a talk co-written with Dr. Elizabeth Unger, will provide a more detailed review of their work on the epidemiology of reproductive health in men and women with ME/CFS. So, you are in a -- for a real treat today. Thank you so much for listening.

Vicky Whittemore: Thank you, Beth. Do you want to go ahead and introduce Dr. Thomas, please? Thank you.

Beth Pollack: Absolutely. So, Dr. Natalie Thomas is a research fellow within the Department of Biochemistry and Pharmacology at the University of Melbourne in Australia. She leads a neuroendocrine -- she leads neuroendocrine research in the Melbourne ME/CFS Collaboration directed by Dr. Christopher Armstrong. She's talking to us today about neuroendocrinology and sex hormones in ME/CFS.

Natalie Thomas: Hi. My name is Natalie Thomas and I'm a research fellow at the University of Melbourne, Australia. And I'd first like to thank the organizing committee for inviting me to share some of my research and thoughts in the ME/CFS Research Roadmap Webinar series in the workshop of Lesser Studied Pathologies. And I've entitled my talk today, "ME/CFS and Neuroendocrinology Evidence, Study Design, and Research Translation."

So, I do think it's worth starting my talk very briefly with reminding ourselves of the broad challenges of studying ME/CFS. And that's to say it's a heterogeneous multisystem illness that involves several physiological systems, including the nervous, the immune, metabolic, and endocrine systems. It's well established that crosstalk between these systems exists in both health and disease. And it's also been proposed that an aberrant state of homeostasis between these systems may be a central causal mechanism of ME/CFS. And so, considering the depth of ME/CFS research in the nervous and immune systems and metabolism, I would argue that the endocrine system is an under-researched area in ME/CFS as both its own entity and as a component of homeostatic regulation.

So, we know that ME/CFS affects people of all races, all genders, all ages. However, the most consistent, credible, predictive risk factor of an ME/CFS diagnosis is being female with a 3-to-1 preponderance. This disparity seems to emerge in late adolescence coinciding with pubertal onset and is then maintained during periods of rapid hormonal changes, so reproductive menstrual cycle fluctuations, pregnancy, postpartum, perimenopause. And this suggests that major endocrine events may be moderating mechanisms underpinning these vulnerability -- vulnerable groups, which of course then implicates gonadal sex steroids.

So, then we want to think about the neuroendocrine system. So, what do we know currently about the biological evidence of neuroendocrine dysfunction? So, this figure allows us to follow the hypothalamus pituitary axis and its endocrine targets, and the broad downstream effects of physiological systems and symptoms implicated in ME/CFS. So, there is evidence for dysfunction in adrenal stress, gonadal sex, thyroid, and renal endocrine systems in ME/CFS including changes in cortisol levels, estrogens, progesterones, T3, T4 thyroid hormones, and aldosterone. And broadly speaking it appears that there's a downregulation of steroid hormone levels and an increase of upstream dysregulation at the level of hypothalamus antibodies, for example, in pituitary antibodies.

So, I'm being very conscious of time. So, I'm only going to go into detail for a couple of examples. And I'll start with the HPA axis output of cortisol. And this has of course been shown to be reduced in ME/CFS particularly in terms of dynamic responses to waking and diurnal variation. And because cortisol can even be defined as one of the most credible biological markers for ME/CFS as it's evidenced at the level of meta-analysis.

And so, this is important in ME/CFS as we know that cortisol output is responsible for diverse physiological roles including increasing energy utilization, including moderating the inflammatory response, and autonomic nervous system so -- activity. So, I think cardiovascular response. And interestingly, lower cortisol levels have also recently been identified as a major distinguishing feature of Long COVID as well. And this is currently being followed up in the literature.

And like seen in ME/CFS, sex differences have been shown in these cortisol levels, although this has been yet to be formally published in the Long COVID literature. So, in terms of the gonadal -- hypothalamus gonadal axis, estradiol levels have been shown to be below clinically normal thresholds in populations of ME/CFS, in addition to lower progesterone levels being shown in the luteal phase in premenopausal women with ME/CFS. And of course, expanding literature is really showing that estrogens and progesterones play a mediating role in the immune and mitochondrial function and neuroprotection, all systems implicated in ME/CFS.

And it's these broad effects of steroid hormones on diverse physiological systems that may speak to both the heterogeneity and the fluctuating nature of ME/CFS symptomatology. And although there is growing evidence of these alterations of steroid hormone secretion in ME/CFS, and certainly enough literature to really think about following this area up, the available research really has severe limitations including either being cross-sectional in nature, which of course entirely ignores the natural course of hormonal fluctuations across the day and month.

And majority of these studies measure only one hormone at a time. And I can't go into too much detail because of time, but the biosynthesis of steroid hormones is very complex. And it's best to look at the network of steroids as opposed to one steroid at a time. And much of this data that I've spoken about in this review and in this talk is actually derived from secondary research questions. So, for example, they've come up in proteomic studies. So, they're not prospective primary aims of studies yet.

And really, in order to understand this research area comprehensively and correctly, there are methodological complexities that we really need to consider. So, on the top right, I'd like to draw your attention to this graph. And what we see here is the change of testosterone and estrogen across the reproductive lifespan for both males and females. And we see that that changes and declines over time in different ways. But what I really want to tell you and point out is that all steroids are synthesized in both the females and males as we can see here, including estrogens and testosterone. And both are important for both sexes, or all sexes I should say.

On the left, you can see hormone levels according to an idolized menstrual cycle of 28 days, where we can observe the changing concentrations of female hormones women throughout the

different menstrual cycle phases. And on the bottom right, we can see the diurnal pattern of cortisol, which typically follows a cortisol awakening response in the morning with then a gradual decline throughout the rest of the day until medium.

And these collective graphs are really here to say, look, if you try and measure estrogens, for example, at different points during the reproductive lifespan or menstrual cycle, you're of course going to obtain significant variability in your data or cortisol at different times of the day. Again, you're going to obtain significant variability in your data. And whilst this may seem obvious, the fact that steroids including cortisol and estrogens and testosterones can affect the immune system and the metabolic response, for example, I would argue that these parameters are critical to consider in much of the ME/CFS research.

And I think this study is a perfect example. So, this study very briefly follows 259 women across two menstrual cycles. And they measured their serum levels of C-reactive protein, of course a protein that rises in response to inflammation, estradiol, progesterone, luteinizing hormone, and follicular stimulating hormone. And they did that up to eight times across a cycle. And what they showed was that CRP level -- levels vary significantly across the cycle. And they also showed that an increase in estradiol was an -- was associated with a decrease in CRP. Whilst an increase in luteal progesterone was associated with an increase in CRP.

This study was performed in a healthy cohort. And this data kind of added to the evidence that endogenous estradiol is -- has an anti-inflammatory effect. But the authors also suggested that there really is a need for standardization for things like CRP measurements to menstrual cycle phase in reproductive-age women. And this of course, is particularly interesting to us in the ME/CFS field as these patients have been shown to have higher levels of blood cytokines than in controls including CRP, again, at the level of meta-analysis.

So, whilst there's growing evidence of alterations in steroid hormone secretion in ME/CFS, the available research as I've suggested has severe limitations. And further, the functional importance has not even begun to be elucidated. And the potential role of this crosstalk between steroid hormones and ME/CFS symptomatology. And then also the downstream mediators of other physiological systems implicated in ME/CFS completely remains unexplored.

So, research priorities for this area of research really begin with the implementation of correct data collection strategies and workflows. So, this includes repeated measure study designs, controlling for biological sex, reproductive phase, menstrual cycle phase, diurnal phase, via a study design. And here we can see quite a comprehensive study design. And this is actually an observational clinical trial we're about to kick off in -- next month in Melbourne, or at least statistically by collecting this information.

We also need to consider the complexities of quantifying steroids as the biosynthesis and metabolism of steroids complex. I'd love to go into detail in more of -- into detail more if I have more time. But basically, to say that we really need to utilize gold standard technologies like mass spec technologies for example, ultra-performance supercritical fluid chromatography rather than using these older cheaper ELISA techniques.

And in fact, we have just submitted an Australian grant in collaboration with Professor Jonas Bergquist, who has developed this technique in his lab. We also need to simultaneously analyze the downstream mediators of the systems typically affected in ME/CFS. So, I'm talking about the immune system, metabolism, kidney function, cardiovascular system. And this is what our study will be doing next that we're kicking off next month.

So, I will end with some questions that are to -- are still to be answered, and they are, are symptoms of ME/CFS moderated by steroid levels? And if so, are disturbed individual steroids or steroid network relationships responsible? Do individual steroids or steroid network relationships associate with other downstream physiological systems dysregulated in ME/CFS in this cross-talking manner? And clinically speaking, do different types of hormonal medications already in use, oral contraceptives, thyroid medications, transdermal estrogens, progesterones, as well as even gender-affirming hormone therapies exacerbate or mitigate symptoms of ME/CFS?

And we end with research translation. Endocrine disorders are eminently treatable. And their diagnosis and management can result in significant improvements in health and quality of life. And so, careful evaluation of the neuroendocrine systems in ME/CFS is an avenue to improve patient's outcomes in a clinical capacity and deserves attention. If high level evidence is found supporting reproductive phases or steroid alterations that improve symptoms, clinical trials, of course, utilizing these steroids like estrogens for example, can be proposed to be trialed in this population via RCTs. And repurposing drugs in the field of ME/CFS is one way we can fast track effective treatments in this population. And many repurposed endocrine drugs have been applied to disease states with beneficial effects including estrogens, progesterone, selective estrogen receptor modulators, which have selectivity for tissues. And very briefly, as I know I've run out of time, I of course love to acknowledge my collaborators and I thank you all for listening. So, thank you very much.

Vicky Whittemore: Thank you, Natalie. That was excellent. So, Beth, can I turn back to you to introduce the next speaker, please?

Beth Pollack: Thanks, Vicky. Emelia von Saltza is a researcher with lived experience in ME/CFS and endometriosis. And has formerly worked in immunology and gynae pathology research at MIT. She's a co-author on our review and reproductive health infections across reproductive health and infection-associated chronic illnesses that I just discussed. She's talking

to us today about endometriosis and ME/CFS.

Emelia Von Saltza: I'm presenting an overview on endometriosis and ME/CFS. Endometriosis, often nicknamed endo, is a chronic, systemic disease where tissue similar to the lining of the uterus grows outside the uterus. It's estimated to affect 10 percent of reproductive-age women and girls globally and unmeasured numbers of transgender, non-binary, and gender diverse individuals. In contrast, one case control study found over a third of women with ME/CFS reported endometriosis. Endo is typically found in the pelvis but can occur throughout the body. For example, gastrointestinal, urinary, and thoracic endometriosis.

Symptom heterogeneity is high and can for example include chronic pelvic pain, severe pain with menses, sub or infertility, painful urination, gastrointestinal symptoms, shortness of breath, coughing up blood, fatigue, and brain fog, which the patient community often refers to as endo brain. Lastly, some endo patients are asymptomatic. Many of these symptoms present or worsen around menstruation and can overlap with ME/CFS symptoms, which also often fluctuate with the menstrual cycle as Beth mentioned and as Dr. Thomas's study.

Endometriosis is a surgical and histological diagnosis. Diagnostic delays can average up to 11 years due to symptom normalization, lack of practitioner awareness or access to specialists, invasive diagnostics, and the gender healthcare gap. These delays may be on average longer for black patients in part due to racial healthcare disparities and racial diagnostic bias in endometriosis specifically. These delays can result in decades of debilitating, untreated pain, which can be exacerbated by the also high rates of delayed and misdiagnosis for ME/CFS.

Symptoms can be severe and isolating, with high financial, social, emotional, and career costs such as lowered school attendance for children and teens, reduction, or loss of career, to name a few. ME/CFS and endometriosis each on their own can be debilitating. And together, that suffering is often compounded.

As Beth shared, last year we had the pleasure of co-authoring a review with the patient-led research collaborative on female reproductive health in Long COVID, ME/CFS, POTS, and connective tissue disorders. Our review quickly became the most viewed in the journal's history with considerable circulation in the patient community. I share this because I believe it speaks to the unmet demand from patients for more research on reproductive health in ME/CFS and other infection-associated chronic illnesses.

Despite the prevalence of a disablement caused by endometriosis, its etiopathogenesis remains largely unknown. The lesser-known bacterial contamination hypothesis proposes that microbes contribute to endometriosis growth and progression via LPS/TLR4 and/or peptidoglycan/TLR2 cascades. Significantly higher levels of LPS and gram-negative bacteria have been found in menstrual blood, peritoneal fluid, and endometrium of endo patients compared to controls supporting this hypothesis. In some cases, antibiotics have been reported to reduce endo

symptoms.

Of all the theories on endometriosis development, I call your attention to this one because the bacterial contamination hypothesis is notable in the context of studies on pathogen persistence, viral reactivation, dysbiosis, and pathobiont expansion in ME/CFS and other infection-associated chronic illnesses. And traction is gaining around this research. The Tissue Analysis Pipeline project at UCSD involving PolyBio is exploring persistent pathogens or infectious processes in endometriosis. And as Beth mentioned, the Tal Research Group at MIT is looking at endometriosis within infection-associated illnesses.

I want to emphasize that endometriosis is a multi-system, multi-organ disease. Confining it solely to guide pathology research and funding limits our full potential for understanding it and its relation to other complex chronic illnesses. Research suggests that endo and ME/CFS share some mechanistic overlaps, which warrant further research. Reduced natural killer cells, cytotoxic function, macrophage alterations, lowered cortisol, elevated oxidative stress, blood clotting disorders, allergies, and mast cell activation are all implicated in both endometriosis and ME/CFS.

Moving forward, increased awareness and screening of endometriosis and ME/CFS research and clinical care will be key in further establishing rates of comorbidity. The extent to which endometriosis predates or predisposes ME/CFS onset versus develops or exacerbates following is under explored. More research is needed to determine directional relationship. It will be critical to develop non-invasive diagnostics for endometriosis, especially to improve diagnostic accessibility for severe ME/CFS patients. Beyond prevalence, it will be critical to further explore patho-mechanisms, in particular infectious processes at the interface of endometriosis and ME/CFS. Thank you all for your time today and to Beth, Vicky, and the members of the Lesser Studied Pathologies Subgroup for their leadership and organization.

Vicky Whittemore: Thank you very much, Emelia. Over to you, Beth, again to introduce the next speaker.

Beth Pollack: Up next, Dr. -- we have Dr. Roumiana Boneva. Dr. Boneva, MD, Ph.D. works in the Office of Science at the U.S. Centers for Disease Control and Prevention. Prior to her current position, she conducted epidemiological research on ME/CFS for 12 years and was a pediatric cardiologist. She's talking to us today about reproductive health in ME/CFS. Dr. Elizabeth R. Unger, MD, Ph.D. also collaborated on this talk. Dr. Boneva?

Male Speaker: You're muted.

Roumiana Boneva: I was muted. Sorry, I was muted. Good afternoon. I am presenting this talk on behalf of Dr. Unger and myself. And I would like to thank the organizers for the opportunity to present at this meeting. Next slide, please. Or can I advance mine? Okay. The findings and conclusions in this presentation do not necessarily reflect the official position of the CDC. And Dr. Unger and I, we have no conflicts to declare. Next slide.

The CDC studies on gynecologic factors associated with ME/CFS were prompted by several previous observations. Most notably, as has been noted throughout these webinars, ME/CFS is more common in women suggesting factors more specific to women could be driving this higher prevalence. However, few studies examined gynecologic history of hormones. These were limited by relatively small numbers and lack of replication. But hysterectomy, endometriosis, polycystic ovaries, uterine fibroids, irregular periods, and galactorrhea were identified as risk factors. Hormones have been even less investigated with one small study finding estrogen deficiency in a subset of women with ME/CFS and another finding higher levels of progesterone and its metabolites. Next slide, please.

CDC examined reproductive conditions in women with ME/CFS that were identified in two population-based studies. The first one in Wichita, Kansas and the second one in Georgia. A few words about these studies. Participants were recruited through a random digit dialing in a geographic area and they answered screening questions in order to identify potential ME/CFS patients and healthy controls. This initial screening interview was then followed by another detailed telephone interview where potential patients and agent sex matched controls answered more questions. Finally, persons who met most criteria for ME/CFS and their matched controls were invited for a clinical evaluation. At the clinic, they responded to a number of questionnaires and had a physical exam and laboratory workup. The final classification of ME/CFS cases and controls was based on the clinical evaluation.

Now, in the Wichita study there was certain -- excuse me, 36 women with ME/CFS. And 48 age matched healthy controls. The mean age in this analysis was 51 years. We found that endometriosis, irregular periods, pelvic pain that was unrelated to menstruation, and reporting gynecologic surgery was significantly associated with ME/CFS. At the same time, the association of hysterectomy and oophorectomy was suggestive. Interestingly, the mean age at menopause and age at surgical menopause were lowering the ME/CFS group than in controls. And the reproductive period for women with ME/CFS was four years shorter than in controls. Next slide, please.

In the larger and more detailed Georgia population-based study of ME/CFS, all these findings were confirmed and expanded. The analysis included 84 women with ME/CFS and 73 age-matched healthy women. The graphs here show the prevalence in percentage of each gynecologic factor in women with ME/CFS, the purple bars, and in control women, the green

bars. Heavy menstrual bleeding was the most common gynecologic problem in women with ME/CFS reported by nearly three quarters. All factors were significantly higher among women with ME/CFS with the exception of removal of ovaries, which was increased but did not reach statistical significance. Next slide, please.

This slide provides more data on the menstrual history of women in the Georgia study. Of note, the mean age at menarche did not differ between women with ME/CFS and healthy controls. However, menopause differed. It occurred on average 11 years earlier in the CFS group at 37.6 years versus 48.6 in controls. As noted before, nearly three quarters of women with ME/CFS reported heavy menstrual bleeding.

In addition, half had bleeding between periods and more than one third reported missing periods. The frequency of these conditions in controls ranged between 22 and 26 percent. In other words, women with ME/CFS were nearly four times as likely as controls to report heavy menstrual bleeding. They were three times as likely to have bleeding between periods and two times as likely to have missed periods. Next slide, please.

Among those with gynecologic surgery, women with ME/CFS were on average five years younger than controls at time of hysterectomy and seven years younger at time of removal of both ovaries. Uterine fibroids were more common in -- also more common in women with ME/CFS. Again, women with ME/CFS who had gynecologic surgery, the relationship between the time of surgery and the onset of fatigue was not clear. However, slightly more women experienced fatigue the same year or after surgery. Next slide, please.

While the CDC studies did not address pregnancy in women with ME/CFS, this is an important question. Studies on how pregnancy and ME/CFS affect each other are very limited. One study found that overall pregnancy did not negatively affect CFS/ME. In that study, 30 percent of patients improved, 29 percent worsened, while 41 percent reported no change. Similarly, after pregnancy symptoms improved and worsened. Symptoms of improvement and worsening were reported equally, each in 20 percent of women. The rest reported no change. As noted in a recent review, more research on this question is needed. Next slide, please.

Finally, we think it's important to recognize the potential impact that ME/CFS has on men's reproductive health. While ME/CFS is more common in women than men, men do suffer with ME/CFS and are often overlooked. We could not find data on reproductive issues in men with ME/CFS despite the indication that this would be possible. Steroid hormones, gonadal, and other, have broad effects on organs and systems that contribute to symptoms of ME/CFS. Men with Long COVID have experienced erectile dysfunction and reduced sperm count. Clearly, this question needs studying. Next slide, please.

So, what we know about reproductive issues in ME/CFS. Information is restricted mainly to women and is relatively limited. We know that a number of gynecologic conditions and events are associated with ME/CFS in women. These include, as already mentioned, excessive menstrual bleeding, irregular periods, gynecologic surgery, early menopause, lower abdominal pelvic pain unrelated to menstrual period, endometriosis, uterine fibroids, and a few other conditions. Therefore, gynecologists need to be aware that women who have had early hysterectomy or have other gynecologic conditions mentioned here may be at risk for ME/CFS. And similarly, physicians need to ask women about gynecologic problems. Next slide, please.

What do we need to know? Many things, of course. But what seems most important is understanding the mechanisms by which these recognized gynecologic and hormonal conditions contribute to ME/CFS onset and symptoms. For example, reduced levels of gonadal sex hormones may contribute to the development or perpetuation of ME/CFS symptoms through several known pathogenetic mechanisms such as impaired sleep, loss of the neuroprotective effects of progesterone and estradiol, increase in pro-inflammatory cytokines and upregulation of inflammation, possibly increased pain perception due to loss of the estradiol's and progesterone's effect on the antinociceptive pathways. That is the hormonal control of pain signaling to the brain.

Also, they may work through impairment of healing and repair mechanisms that are mediated by estrogen and progesterone and other gonadal hormones. On the other hand, triggering events for ME/CFS such as infection or autoimmunity could mediate gynecologic conditions. Next slide, please.

Finally, listing research priorities in reproductive health is challenging because there are so many questions. We propose this list and welcome amendments. We suggest that to identify underlying mechanisms, we need to do some groundwork. First and foremost, studies need to systematically collect detailed gynecologic and reproductive history. We need to systematically determine steroid hormone levels and how they correlate with symptoms all the time. It is also important to include sex and gender in all analysis. In particular, analysis of steroid hormones in women need to -- in particular, analysis of steroid hormone -- hormones in women need to account for and stratify by phase of the menstrual cycle, menopausal status, use of hormone therapy.

Such stratification may be needed not only in endocrine studies, but in all studies. It is time also to study the impact of ME/CFS on male fertility and sexual function, as well as the relationship with hormonal levels. It is necessary to study the impact of pregnancy on ME/CFS and vice versa. And also, pregnancy outcomes in women with ME/CFS. And last but not least, it is important to look for overt of subclinical genitourinary infections and study their role as well. With this, I can conclude the presentation. Thank you for your attention. And again, thank you

to the organizers for -- thank you to the organizers of this webinar. Now, Dr. Unger and I can take questions.

Vicky Whittemore: Yeah. Thank you very much for that presentation. There's a comment in the Q&A. What diagnostic criteria were used and was PEM a requirement for the individuals that were -- post-exertional malaise a requirement for the individuals that participated in the study -- in the two studies?

Roumiana Boneva: So, the two studies --

Elizabeth Unger: I would like to answer that if I could [laughs].

Roumiana Boneva: Okay, Elizabeth.

Elizabeth Unger: Yeah. Yeah. And indeed, these were studies that were conducted some time ago.

Vicky Whittemore: Right.

Elizabeth Unger: And the case definition that was used was the 1994 research case definition. And post-exertional malaise was not a requirement. But the -- but more than 80 percent of the patients had post-exertional malaise just as a part of the fact that these symptoms are so closely related.

Vicky Whittemore: Great. Thank you. And so, I guess I would ask for your suggestions about how to move forward with expanding studies on reproductive health in both men and women.

Elizabeth Unger: Yeah. I mean, I think the common data elements idea is a great one.

And just reminding investigators to ask these questions. And it is easy when you're focused on so many other problems. And, you know, I think today's lesser studied pathologies have been very interesting in just showing how tightly integrated everything is.

Vicky Whittemore: Yeah.

Elizabeth Unger: And so, patients get fatigued answering every, every, every question. Nonetheless, unless we find out what the gynecologic events are and the sexual health issues are by asking questions, there's no way to come up with the data. And so, I think by strategically sort of timing giving questionnaires, in other words, not giving them all at once, being as focused as possible and, you know, sort of skip patterns. So, if you don't have anything, you can just

kind of skip all over the area. But it is just striking how often we just don't have the information. And then when the information is there, it's often not the focus of the paper so it's hard to find it.

Vicky Whittemore: Yeah.

Elizabeth Unger: So, I would just say that researchers, you know, include a term if they've -- in their keywords to be sure that it will come up. So, if you have looked at reproductive factors at all, be sure that that is included as an index term, because finding the literature is hard. Yeah.

Vicky Whittemore: Right. And to extend that comment, I think even just analyzing data separately, male versus female.

Elizabeth Unger: Males and females, it's absolutely -- at least --

Vicky Whittemore: Which we're seeing with a lot of the data coming out of the Hanson and Unutmaz and Lipkin centers is critically important.

Elizabeth Unger: Right. At least looking at it stratified and then if there's no difference, then merging.

Vicky Whittemore: Right. Right.

Roumiana Boneva: And for women, analyzing any data would be important to account for the menstrual cycle phase in pre-menopausal women as Natalie Thomas nicely showed with the illustration of hormonal levels.

Vicky Whittemore: Yeah. So, we're running a little bit behind schedule. I think we'll take a quick 5-minute break and then come back for a panel discussion. So, I invite all of the speakers when we come back in, let's say at 2:35 p.m. We'll give you 7 minutes, 8 minutes. So, let's come back and then we'll have a panel discussion with all of the speakers. Thank you.

Vicky Whittemore: I'd like to welcome everyone back from the break and ask all of the speakers to please turn your cameras on. I'm going to turn it over to Beth to start our discussion. Go ahead, Beth.

Beth Pollack: Thanks, everyone. So, we're looking forward to this discussion with all of our speakers. And one of the first questions we wanted to start with is, what are the most important clinical studies that you would like to see for your topic that can help us advance towards clinical trials on treatments for your topic? Anyone, chime in.

Julie Rehmeyer: I mean, I think for my topic the place to start is just figuring out how common it is. You know, I think we kind of need that to build on. You know, again, at this point, we really only assess patients for CCI if they're really severe. Because we're only thinking about it in the context of possible surgery and who's going to sign up for neurosurgery unless they're really bad off. But that doesn't mean it doesn't occur in patients who are less severe. So, you know, just having -- like doing cervical MRIs and seeing what the measurements are for a spectrum of patients would be, I think an important place to start. Not going to get us to clinical trials but prediction.

Beth Pollack: I also just want to add on, Julie, that there may be some indicators that are not necessarily imaging based. And Dr. Ruhoy can talk about this, that we can incorporate into our clinical studies to try to assess for spinal conditions and some of these other less studied pathologies. And I reviewed some of those in our -- in the first talk because we are including them in our clinical study at MIT to try to assess for as many of these pathologies as possible.

Julie Rehmeyer: Yeah, I know.

Ilene Ruhoy: Yeah. You screen for CCI, whether no matter the severity of the disease, I get MRIs in -- on cervical spines for just about every patient. And I do the measurements and I very often find CCI doesn't always mean that it's significantly contributing to the symptoms. But when it does exist on any level, we talk about options that are -- I mean, I have more conversation -- conversations about non-surgical options than I do about surgical options. But obviously, if it's severe and if we -- if I think that it's contributing greatly, then we move forward with the invasive cervical traction. We don't even talk about surgery until after the ICT. So, it's a regular part of the workup at this stage for all patients.

Beth Pollack: Dr. Ruhoy, are there certain non-surgical treatments that you hope to see more research on in the future -- potential treatments?

Ilene Ruhoy: Yeah. I mean, one of them that I touched upon earlier with one of the questions that Vicky had asked me, was about physical therapy. I think that there are a lot of particular

exercises that can help strengthen some of the ligaments and create more stability of the cervical spine. But also, we do know that the more inflammation that there is, the more laxity of those ligaments exists. And so, the symptoms of CCI can be -- can potentially be worse. And so, if we reduce inflammation, then the goal, of course is to reduce the burden upon those ligaments and upon the connective tissue so that we can minimize the severity of the CCI.

And so, we do a lot of work with regards to mast cell stabilization, for example, a lot of anti-inflammatory work, a lot of reduction of immune responses and whether it's to pathogens or to things in the environment. We work to bring down inflammation. And we know just from all the patients that we've worked with, that the more we can bring down the inflammation, the better these patients do with their CCI. So, there are non-surgical means of managing CCI. Unfortunately, it's not curative. The instability will continue to exist.

And again, based on level of inflammation, it can wax and wane in terms of severity. But it does -- it doesn't resolve. You know, and that's the problem. It still is, at its very core, an anatomical diagnosis. And so, again and at this point in time, the only way we really know to correct an anatomical misalignment of any sort is really with surgical intervention. Some surgeries are, you know, not as severe as others, of course. But we can reduce the pathology of it for sure, and the disability. So -- and that's our goal ultimately.

Julie Rehmeyer: Just a couple of thoughts on that. One is, so I -- you know, I went into remission in 2013 with extreme mold avoidance. I would be very surprised if I did not have CCI to some extent at that point. And then when I got subsequent exposures and more inflammation, the CCI got so bad that I couldn't have a reasonable quality of life without addressing the CCI. But that reinforces what you're saying that, you know, if we can manage the inflammation, if we can control the MCAS then, you know, we may be able to keep the CCI not a huge problem even if it's there.

Ilene Ruhoy: Yeah. I 100 percent agree. And that's what Dr. Maitland and I are always talking about. About ways that we can manage these patients without surgery by just managing the mast cell activation and any other inflammatory response. And yeah. And as your experience supports that, you know, it does really help.

Julie Rehmeyer: And then the other thing that I just wanted to say is in terms of non-surgical management techniques is Alissa Zingman at PRISM, you know, has a whole kind of set of techniques. And I've never been to her, I don't know what they are. But I've heard reports from other patients that her techniques kind of go beyond standard physical therapy and can be profoundly helpful in management issues.

Ilene Ruhoy: Yeah. That is true as well. She's -- she has a three-week rehab program for CCI.

And I actually refer a lot of patients into that program. And it is incredibly helpful. It is a successful program. Again, I don't know yet, and this is maybe an area of research, you know, will it obviate the need for surgical intervention years from now? You know, I don't know the answer to that question. But I agree with you, it's a remarkable program.

Beth Pollack: And so, just --

Julie Rehmeyer: And then a quick question for you, Dr. Ruhoy. When you screen patients for CCI, what would you say about how common it is?

Ilene Ruhoy: It's very common. So, there's a severity of CCI. So, you know, most patient -- I mean, I would say that most EDS/ME/CFS patients have some degree of CCI. It can be mild. You know, sometimes the morphometrics are just borderline and the ICT is sort of equivocal. And so, it can be mild. It doesn't have to be the reason for everything. So, it very often exists though. Very often.

Beth Pollack: I just wanted to say --

Anne Maitland: If I may, I just would like to chime in regarding the crosstalk between the autonomic nervous system and the mast cell compartment. Because we've seen significant induction of tolerance in the mast cell compartment, even using vagal stimulation. So, I think it's important to kind of understand that when the cervical spine and the -- and also, the lumbosacral spine is involved and you have individuals that are -- have been deconditioned for so long there's a lot of a lack of resources to help stabilize that musculoskeletal system, which in turn will influence the balance between the vagus and the adrenergic system.

And the mast cell compartment, we know. Like, I used to make mice allergic to cheese, a certain species of mice, if I traumatize their neck and then introduce the milk. So, I would say the same thing for you, Julie, when, you know, you -- when you start to deteriorate, then, you know, the autonomous system is imbalanced which will then alert the mast cell system. So, now you have autonomic stimulation. And now, the mast cells are, like, looking for all usual suspects now, especially something like mold.

So, I think it -- I really think we can't address this in silos. It really is a crosstalk that we really do need to appreciate and that's why we have to do these studies that, you know, what we were doing. Laura and Ilene and I have been talking about this for a long time. It's really important to try to understand the structural, the immune dysregulation, and the autonomic dysregulation in order to help people not necessarily resolving with surgical approaches.

Elizabeth Unger: So, switching the topic a little bit I think that we did, in response to Vicky's

question, sort of indicated that basic epidemiologic data on the gynecologic history should be included. But I have a question for Dr. Pace about the microbiome particularly indicating that you think the importance of the small bowel microbiome is. And how could that be mediated and how are you proposing to study it or how should we -- it be studied?

Laura Pace: Yeah. It's a great question. I think that there's a lot of interest in, like, microbial communities contributing to disease, right? But a lot of it's very correlative, not causative. A lot of it's been -- most of it, 99.9 percent has been done on stool. But when we do look at microbial community structure from different areas in the gastrointestinal tract, they're really different. And so, it matters where we're looking.

And so, I really think that we need to start to focus a lot more on the small intestine in the different regions of the small intestine, again, because that's where they're having the greatest interaction with the immune system and the nervous system directly. And it's just -- it's more permeable there, and it has to be because of the function of the gastrointestinal tract there. And so, there are tons of tools that we can use where we can sample the community structure.

And we need to do three-dimensional spatial mapping because that degree of community structure actually matters. And we know that from environmental studies, like looking at hot springs and things like that, that the microbes themselves, how they organize are signaling to each other and sort of changing sort of community structure metabolism. And so, it's not going to come down to one species, it's going to come down to community structure and that sort of collective like metabolism and activity.

And so, I think my personal opinion is it's premature to be like, we need to supplement this one microbe back into the system. These are complex systems. They're also systems that our immune system has developed some degree of tolerance for. So, our microbial community doesn't raise alarms for our immune system if it's sort of imbalanced. But then when we start to add maybe organisms that weren't previously in our community or at levels that shouldn't be there, are -- it's another signal to our immune system. And so, these alterations that are being proposed I think are premature for our level of understanding given the complexity. And we know this from other areas of microbial ecology.

Anne Maitland: May I add that there's an organ system that really doesn't get enough attention, and that's the respiratory tract. As much as I adore learning about the GI --

Male Speaker: Thank you.

Anne Maitland: -- especially when you talk about SARS-CoV-2, Candace, PANS, ME/CFS following upper respiratory infections, I think it's also important to understand that so many

people are running around with undiagnosed respiratory disease. The dysbiosis we have seen in individuals who even have something like selective immunoglobulin A or selective anti-polysaccharide antibody deficiency, so enough to cause -- enough dysbiosis to cause that chronic inflammation in the respiratory tract possibly also contributing to the GI tract as well.

And then that allows that ongoing permeation of foreign entities into the subepithelial layers and causes that chronic engagement, not only of the immune system, but also the somatosensory networks. And so, it's really interesting to see what happens when you start trying to restore balance regarding the connective tissue around the immune system. And then also neurologically such that it's a coordinated effort between all the disciplines. This has to be a team effort. It really does.

And this is what the beauty of this -- of all of us sitting down together in order to kind of share our observations and not just stick to just one organ system. I think it was Dr. Ruhoy who mentioned, and also Dr. Pace, that it's important to have multi organ system, you know, focus. We've got to get out of the weeds and kind of go to the level of description biology.

Roumiana Boneva: I think if I can say something. I think what the patients need the most is a complex assessment and evaluation and looking at them as a whole. Because what's happening is they can go from clinician to clinician, and everybody looks at their subset of illnesses. And often things are so connected in our body. Like, we've seen in our studies, we did heart rate variability studies, we see that the sympathetic system predominates. Which means that the vagal is suppressed and what can we do about it? And when you said -- when you mentioned vagal stimulation, what methods do you use?

Anne Maitland: So, we've employed IV stimulation of the ear, acupuncture, acupressure. There's also targeted physiotherapies will also do it. So, it all depends on what the patients will tolerate. Some actually -- you know, it -- it's interesting. Like, I find it fascinating for instance, that acupuncture points are chock full of mast cells, sitting right next to the nerve clusters.

Roumiana Boneva: Yeah, this was very interesting on the presentation. Yeah.

Anne Maitland: Right? So, I think -- and we've seen individuals. So, here's the thing, if for anesthesia, if you actually place cromolyn at the site of the acupuncture site, you actually interfere with the analgesic effect. So, this is not -- this is, again, not a one trip. This is all about what is all of those organ system seeing there that they're willing to steal resources from elsewhere in order to fight what it might think is an impending danger.

And then once that impending danger is perceived to be gone, then you're willing to start shifting resources back to just, you know, daily metabolism. And so, I think it's a very fluid and

understandably so because, you know, again, what we breathe in, what we ingest, what our skin is dealing with -- which, by the way, all of those organ systems are at toxic effects because of what we wear, what we eat is stripping the linings. And that's helping to expose the somatosensory network as well as the components of the immune system that are sitting right in that line as well. So, this is -- and all that we're seeing from an immunological standpoint has only happened within the past 30 years.

Laura Pace: I want to go back to Dr. Boneva's point about sort of these in-depth, you know, analyses. And that was one of the points that I was trying to make, is that we really need deep clinical phenotyping on these patients so when we have these studies, we don't have questions about, well, did they actually have this too? And so, I mean, I really think that we need to change the way we think about ME/CFS. It's a very marginalized condition, and I know I'm speaking to the, you know, to the choir here.

But I mean, there we have to move with urgency. We have centers of excellence like in the oncology space. We need to develop these centers of excellence clinically. We'll be able to collect the data that's necessary. You know, people are asking, like, "How do we prevent the natural history of these conditions from getting worse?" If we identified these patients earlier, intervened more aggressively earlier, we wouldn't be seeing these severe cases.

And so, I think because the patients look fine, no one is really like -- at least on the clinical side, there are few of us, right? But, like, also we're marginalized too within our clinical practices, often because we take care of patients like this. But I mean, we have to change the thought process and really move with urgency to develop centers where patients can come to and get this comprehensive care, so we're not waiting, and they're not traveling all over the country. Kind of collecting specialists that do this one piece and we're all working together and learning from each other at a faster rate. And I just think that has to happen.

Vicky Whittemore: Yeah, I'll chime in. I absolutely agree with that. So, that's really happened quite a lot in the rare disease space where multidisciplinary clinics have come together to really provide. Because, you know, these, especially children with rare genetic disorders, will present with a symptom that will then manifest in other ways and lead to the need for multidisciplinary care. And if you can come to one center and get that care, the care you need from those specialists in a coordinated way, it makes such an incredible difference. Absolutely agree with that. So, Beth?

Anne Maitland: If I could just --

Vicky Whittemore: No. So, go ahead with the information.

Anne Maitland: If I could just echo on what Dr. Pace was just saying. You know, I agree those centers are not only important for the diagnostic and management, but that also we need to educate the practitioners who see these patients in the first place. And that has to start at the level of the medical school because a lot of these students are getting educated in very siloed approaches and are not taking a broader impact.

And unfortunately, whether we're talking Canada or the U.S., I can't speak about other organization or other countries, but it's a 5-minute rule. Like, the average patient has 3 minutes, 3 to 5 minutes to stake their claim. There's no talking multi-organ system. You have to pick what bothers you the most. And so, it's a very siloed approach. So, you need -- and I think this would be these centers, is you have the general practitioner who's acting as the co-pilot of the patient to help navigate this very rugged terrain and then knows who to send to as opposed to people guessing for the most part. And then -- and still everything is very siloed.

Vicky Whittemore: Well, the other thing, having very well phenotyped individuals with ME/CFS would do, is to then provide the cohorts for clinical -- targeted clinical trials, which would absolutely facilitate research all the way around. So, Beth, I see you've gone off camera. Do you want -- we've got 5 minutes left. Do you want closing remarks?

Beth Pollack: Yeah. Sure. I have closing remarks slides if someone wants to bring them up. Great. And I just wanted to say in our -- just based on our last discussion, I think it'll probably be important to publish more case studies on interesting treatments even before we get to the clinical studies. There's a real need for case studies across emerging and innovative treatments in the field. Okay. Closing remarks. So, thank you so much, everyone, for coming to today's webinar. We are so grateful for the incredible speakers that we had today who are leading experts in these less studied topics. We really appreciate your support in the Q&A and involvement as viewers and just members of the ME/CFS community.

I'm going to briefly overview the topics that we discussed in this webinar. We discussed connective tissue disorders in ME/CFS, which I and Dr. Ruhoy and Dr. Maitland touched upon. We discussed spinal and mechanical disorders in ME/CFS, which Dr. Ruhoy and Julie and I discussed. And unfortunately, Dr. Klinge couldn't make it today. Dr. Maitland discussed mast cell activation disorders. Dr. Pace discussed gastrointestinal conditions and neurogastroenterology. And Dr. Thomas discussed neuroendocrinology and sex hormones. And then reproductive conditions and reproductive health were discussed by Dr. Boneva, Dr. Unger, myself, and Emelia von Saltza.

We also talked a lot about the crosstalk between multiple pathologies in ME/CFS because it's an illness that impacts many organ systems. This is a complex illness whereas several of our speakers pointed out patients with some of these less studied pathologies are falling through the cracks of both research and healthcare in some cases. I want to reiterate from my first talk today that there are other less studied pathologies that we weren't able to cover today. And that ME/CFS in general necessitates more research including these topics and the topics covered in the other webinars in the series.

Co-organizing and envisioning this webinar and proposing and discussing the topics and speakers and chairing the Less Studied Pathologies Subgroup over the last year has been deeply meaningful. As I mentioned in my first talk, this webinar wasn't always planned. We created it because we saw a real need to discuss some of the less studied pathologies that greatly impact subsets of patients with ME/CFS. And that didn't really fit within the other webinar topics.

And this webinar in some ways marks an important event because to my knowledge it's the first time that some of these pathologies, like connective tissue issues and spinal conditions and possibly an in-depth discussion of reproductive health in ME/CFS have been discussed like this in an NIH event or similar high-profile ME/CFS event. So, thank you, Vicky, and thank you to the NIH for providing us with this incredible first of its kind opportunity to discuss and highlight both what we know and the research gaps and priorities on these underdiscussed topics that severely impact many ME/CFS patients.

So, I'm going to try to work tight for time. So, I'll try to rush through this. But in terms of next steps, in my first talk today I outlined some of the ways research groups, including our MIT Tal Research Group, are already incorporating these lesser studied pathologies into research. And as we move forward, it will be important to consider next steps. Here are some thoughts, somewhat general because they go across topics. I truly hope that this is the beginning of a larger collaborative discussion in our field on these and less studied pathologies and ME/CFS in a discussion that spans scientists, patients, advocates, clinicians, and really the whole community.

So, can we study risk factors and biological mechanisms of these less studied pathologies? Who is at greatest risk for them? How might these pathologies, if they're preexisting, pose a risk for ME/CFS? In terms of risk factors, Dr. Pace discussed the importance of genetics research. In our reproductive health talks, we covered how several reproductive health conditions are risk factors for ME/CFS. Additionally, a common but less discussed trigger preceding event of ME/CFS, are physical accidents like injuries, falls. Could this perhaps have something to do with the spinal conditions or connective tissue disorders? Could that play a role in this? Dr. Ruhoy and I both discussed very high rates of comorbidities and overlapping symptom -- symptomatology between connective tissue disorder, spinal conditions in subsets of ME/CFS patients. So, what subsets of ME/CFS developed these pathologies?

Something that I think a lot about -- I think several of us think a lot about, is: are there many roads to Rome? In the sense, can we better understand how different types of illness and immune triggers, pathomechanisms, and pathologies, including the less studied pathologies we discussed today, contribute to the symptomatology of ME/CFS. Importantly, what can these less studied pathologies tell us about different disease trajectories within ME/CFS and phenotypes both within ME/CFS and phenotypes across this group of frequently co-occurring chronic illnesses?

How might these phenotypes inform both precision medical treatment and research directions and potentially clinical trials as we move forward? How can we look at ME/CFS in a way that includes the complexity of how it connects, co-occurs, and overlaps with the less studied pathologies that we discussed today, the common comorbidities. This is a main focus of my research, and it's also the focus of many of the people's research and clinical practices who spoke today.

Can we explore the role of chronic inflammation, immune dysfunction, and pathological immune responses to infection in some of these pathologies? Does this present a potential pathway for therapeutics? So, for example, today several of us discussed research and how chronic inflammation can directly damage connective tissue and is suspected of contributing to these spinal conditions. And also, Dr. Pace touched on inflammation and neurogastroenterology. Emelia and I discussed how emerging marine research suggests that infections such as

pathogenic bacteria and immune responses to it may be part of the etiopathogenesis of endometriosis.

More research is needed, but if a third of female ME/CFS patients are developing endometriosis, this is an important topic to discuss. One question is, can we understand the immune checkpoints or immune regulatory checkpoints that can be potentially targeted therapeutically? This is a topic that Dr. Mikki Tal and we at the Tal Research Group discuss regularly. So, for example, with endometriosis or tethered cord syndrome, could we treat them sometimes non-surgically? In the future, are there immune therapeutics that could even prevent re-tethering?

Unfortunately, Dr. Klinge couldn't be here to talk about her findings on inflammatory cell invasion and important tissue changes that she has been finding in the phylum of neurosurgical EDS patients with tethered cord. Could we develop treatments to help prevent many of these pathologies? So, we can think -- one thing I hope we think about taking kind of moving forward from this webinar is prediction, prevention, and non-invasive treatments.

Another aspect is earlier diagnosis. Many of these less studied pathologies have lengthy, average diagnostic delays. As Emelia mentioned, up to 11 years for endometriosis. For hEDS, a quarter of European patients report that it took them over 28 years to be diagnosed. And 20 percent of these patients saw over 20 doctors until diagnosis, with half of them reporting being misdiagnosed. So, all of these pathologies have lengthy diagnostic delays. In order to improve research, it would be great to improve diagnostic accuracy and reduce those diagnostic delays.

And another thing to consider is the translational impact of this research. How might studying the less studied pathologies inform and improve healthcare and medical education so that complex patients with these pathologies don't fall through the cracks? As we've just discussed, we need comprehensive clinical care for these complex patients, that breaks down the silos and takes into account the heterogeneity of ME/CFS and the less understood aspects of this illness.

Integrating these topics into ME/CFS research will be important. As we discussed, there exists a paucity of research on many of these topics with many outstanding questions. And additionally, one thing we can do is start to screen for them. Within our research, I listed ways we can do that in our first -- in my first talk. It goes beyond medical history because many patients are undiagnosed, so validated surveys, diagnostic questionnaires, in-person assessment, and other types of testing and imaging.

The opposite could also be true. Can we include ME/CFS cohorts in studies traditionally outside this field, but focus on these less studied pathologies? This is especially pertinent for reproductive health conditions. And perhaps moving forward, can we form collaborative research working groups in the field to advance research and understanding of these less studied

pathologies? Continue -- to continue this dialogue, I hope to start collectively an interdisciplinary working group in the field on the development and progression of connective tissue and spinal conditions across comorbid infection-associated illnesses to help identify research priorities. Stay tuned for 2024.

And moving forward, could we also include discussions of these and other less studied pathologies in research conferences and webinars like this one? This leads me to just the acknowledgements. As I mentioned, co-organizing this has been incredibly meaningful. I hope that it'll be impactful and lead to further discussion and hopefully advancements in research. This is my email. Feel free to reach out.

Thank you to all of our incredible speakers. I want to say a big thank you to the members of the Less Studied Pathologies Subgroup who have been committed and thoughtful and caring about creating this special webinar. Thank you to Vicky and also to Holly and to all of the people at NIH who have made this happen. Thank you to the Tal Research Group and to Mikki Tal who leads it. And thank you so much, especially to the ME/CFS community, the patients, the clinicians, the scientists, the advocates who really make this community something very special and help us to move it forward, hopefully into studies on treatments. Thank you so much.

Vicky Whittemore: Well, thank you so much, Beth. And with that we'll close the webinar. There were so many excellent questions that we couldn't get to. We'll try to capture them and to find a way to really address some of these really excellent questions. I think this was probably our -- one of our best attended webinars with at -- I think with the peak we had over about 300 people on. So, that's really fantastic. So, thank you very much, Beth, for all your work to put this together. Special thanks to all the speakers and special thanks to all their participants for making this a really successful webinar. Thank you very much.

Beth Pollack: Thank you.