Transcript

Jeran Stratford

Hello, I'm Jeran Stratford from the RECOVER Administrative Coordinating Center and the moderator of today's webinar. Welcome to the RECOVER Research Review or R3 Seminar. The goal of this webinar series is to catalyze a shared understanding of the research within the RECOVER Consortium. The topic of today's seminar is Findings From Autopsy Studies. It is important to note that this seminar series is focused on scientific research and is not intended to provide any clinical guidance. I want to start by thanking everyone who submitted questions beforehand. Please submit any questions that arise today using the Q&A function in Zoom. After the presentations, we will answer as many questions about today's topic and presentations as possible. Some questions may also be answered within the Q&A. An FAQ document from this seminar will be posted with the recording of the seminar on recoverCOVID.org. It will include the answers for questions relevant to the seminar that were submitted in advance for today.

Questions about other scientific topics will be addressed in future webinars and answers to broader questions about RECOVER will be answered in the faqs@recoverCOVID.org. Today's speakers will discuss what they have learned about PASC from autopsy studies, the gaps in our knowledge, and how RECOVER will contribute to filling these knowledge gaps. Today we will hear from Stephanie Haasnoot, she is a member of the Pathology Family Liaison Group at Mount Sinai Hospital and the RECOVER Site Coordinator handling screening, enrollment, family outreach, and data collection. Stephanie has experience in evidence collection, histology, procurement and decedent management through her work on the special operations unit that trained Army Air Force National Guard in decedent affairs that were deployed in New York City. Dr. Lauren Decker is a Board Certified Forensic Pathologist and Assistant Professor of Pathology and Forensic Pathology Fellowship Director at the University of New Mexico. She is an autopsy cohort site PI utilizing postmortem computed tomography to study SARS-CoV-2 infection.

Dr. James Stone is the Director of Autopsy Service and Head of Cardiovascular Pathology Service at Massachusetts General Hospital and an Associate Professor of Pathology at Harvard Medical School. Dr. Stone's research has focused on describing the spectrum of cardiac changes associated with SARS-CoV-2 infection. Today we will also be joined by Dr. Marie-Abele Bind as our discussant. Dr. Bind is an Instructor of Investigation at Massachusetts General Hospital Biostatistics Center. Her research interest focus on developing causal inference models for quantifying the effects of randomized and non-randomized exposures on various outcomes and understanding the mechanisms explaining these effects. And with that, I will turn the time over to Stephanie.

Dr. Stephanie Haasnoot

Hi everyone. Thank you for joining. I'm going to go over today the importance of the autopsy conversation. My time at Mount Sinai has been focused on the RECOVER project, while also assisting families and assisting the autopsy service and acting as kind of a bridge between conversations with patients, their families, and the clinicians. Next slide. So I like to go over medical ethics, family dynamics, and the importance of body donation specifically for RECOVER. So my talk will be about RECOVER project itself, the study goals and inclusion criteria, steps involved in enrollment, the importance of ethics, engaging and navigating family dynamics in the consenting and autopsy process, and the importance of body donation itself. Next slide. So what is RECOVER? RECOVER is a research project that aims to learn about the long term health effects of COVID, also called long COVID. It's paid for by the NIH, the National Institute of Health.

And our goal is to better prevent, test, and treat long COVID in the future. A few of our guiding principles are that we are patient centered and participants are our partners. It is a national study with a diverse population. It's a multidisciplinary collaborative teamwork and it's adaptive. We have approaches based on emerging science and being able to make changes to treatments based on new findings. The research cannot function without patient participation and it is important to keep that in mind as clinicians and researchers and respect the individual that is either donating their time and energy for the adult cohort or donating their organs and body while also remembering that the family left behind is affected as well. Next slide.

A few goals of the study is to identify, evaluate, and characterize the clinical manifestations of long COVID in adults who die after infection. We are identifying risk factors related to long COVID while also considering differences in clinical manifestations based on sex, race, and ethnicity. And we're trying to develop accurate quantifiable measures for long COVID. By developing these definitions, clinicians will be better equipped to care for patients who are still living with long COVID and by giving it a name allows patients to know that their symptoms are being taken seriously and they're not alone in their suffering. So some of the inclusion criteria for this study is that the decedent must be 18 or older. They must have had suspected, probable, or confirmed COVID with or without long COVID. So a COVID infection at any time in the last few years will qualify them for the study, but they must have died 30 or more days after a symptom onset for it to be considered long COVID. So an acute infection within 15 to 30 days prior to death is not considered long. Next slide.

So now I'd like to just go over some steps involved in enrollment and then some just data on the study itself. Next slide. The team involved in the RECOVER Tissue Pathology Study most importantly is the decedent. They're making the ultimate contribution to science and the ultimate sacrifice. And in that group I also like to remember that the family is affected as well and that they are the ones who we are discussing all of this with once somebody has passed, and they also just need to be remembered that they play a key role in this as well. The authorized decision maker or the legal next of kin is the one who gives consent to enroll the decedent into the study. The site coordinator, such as myself, screens the decedent electronic medical record for eligibility and then

enters that data into REDCap. The pathologist team, they perform autopsies and collect biospecimens for the PASC Biorepository Core. The pathologists and the rest of the team also prepare and ship biospecimens to the Mayo Biorepository.

The neuroradiology teams perform the brain and CNS MRIs on select decedent samples and the pathologists also assemble all data and enter their final report into REDCap. Next slide. So just to touch on the steps involved in enrollment for anyone who's not familiar is that once a potential candidate is identified through screening of the electronic medical record or speaking with families, the screening confirms their eligibility. The PI and the clinician are notified. Once decedent is pronounced, the legal next of kin is approached. Consent would preferably be obtained. Decedent body is then transported to the autopsy suite where the autopsy begins. We intend to do this within 24 hours of somebody passing because we want to respect the family's burial rights and traditions and allow them to still move forward in their grieving process. Specimens are collected, prepared, stored, and shipped. The autopsy is completed and the decedent remains are returned to the family, meaning that they either request for burial, cremation, city burial, whatever the family's desires are is where the decedent will go. And then the electronic medical research data is entered into REDCap.

Next slide. A few things for site management is that we have a few daily efforts to stay on task and to stay in our path on the study. And we do daily screen for potential candidates. We have weekly reviews of the enrollment barriers, things that are preventing us from enrolling, things we can do better. We have team reviews of our current enrollment targets and how far along we are on that scale. We do daily REDCap data entry. Combing through medical records does take a lot of time and we want to make sure that we're thorough, so it does happen on an ongoing basis. And then we also follow up with the PASC Biorepository Core for shipment information and specimen accuracy just to keep everybody involved in this nationwide study on the same page. Next slide.

So this is where we get into kind of the bulk of my responsibility and the things that I actually care for very much is engaging and navigating the family dynamics that happen when discussing autopsy and tissue RECOVER. Next slide. The most important part about approaching a family is knowing who to speak to. So determining the authorized decision maker for an after death donation is important for the clinician, or the psych coordinator, or anybody involved to determine before approaching a family member. This authorized decision maker, the legal next to kin, is not necessarily the same person as the healthcare proxy. And a lot of families don't know this and it's not by any fault of theirs, but it is just important for the clinicians and coordinator or whoever's obtaining consent for each site to be familiar with their state laws on legal next of kin. This legal next of kin is the same person who authorizes organ donation or for transplant.

So if you needed to speak with the anatomical gift conglomerate of your state, they would also know better on how to approach families and who is the legal next of kin. And it is important when approaching these legal next of kins that we are compassionate and we can explain thoroughly to a family member why they may have been healthcare proxy but that terminates at time of death and that they're no longer the legal next of kin

because it is a very delicate subject. So if we can go into it with compassion and knowing that this is not public knowledge and not something that everybody just assumes, then it'll help the conversation move along. Next slide.

So once a patient is admitted to a critical care unit, it is understood that they're receiving end of life comfort care. Families are made aware of this by the clinicians and they're often told they should prepare themselves time to say goodbye, time for other family members to travel to see their loved one, and start the process of making necessary arrangements. So although this is challenging for clinicians and site coordinators to approach, this is the ideal time to begin conversations about organ donation, autopsies, and any research projects their hospital is affiliated with. This will give patients and families time to consider their options and be able to make decisions together. Sometimes this is also helpful for the patient themselves to be involved in the conversation and that they have a say in what happens to them or maybe they have strong opinions on if they want to donate their organs or a research project they're familiar with.

Waiting until late care stages and while families are grieving is really doing a disservice to the families and the patients themselves. At these late care stages, the options of autopsy should be discussed and it allows families the time to come to terms with things before they're grieving and they don't have to make these final decisions while grieving, while just losing their loved one, because a lot of these studies they have time constraints. Families deserve the right to be made aware of all their aftercare options and an autopsy is an aftercare option. So a reluctance from clinicians or researchers to even discuss autopsy or research opportunities in general denies the patient and the family their opportunity to participate in ways that can be very meaningful for them. Continue.

So in doing so in approaching families, we do need to keep in mind doing so with an ethical approach and coming from a place of compassion and not necessarily just scientific cold answers. Next slide. When families feel that researchers or clinicians may not be forthcoming with information or may not have answers that they're looking for, they can look elsewhere and the elsewhere, such as the internet, can be full of misinformation, hearsay opinion, and especially living in this diverse digital age where people get information at the tips of their fingers. It's important to be honest and forthright when speaking with families, even if we think it's hard for them to hear and the answer's not going to be what they like, they deserve the proper direct answer and especially during a difficult time. So we do ask that all the clinicians involved in the RECOVER study or any research study available at their sites are familiar with the requirements and that they know the steps involved in enrolling a decedent or speaking to a family so that when families do have questions, the answers are right there for them.

So the internet, while it's a good source to disseminate information, it does have its downsides and it can turn into a plethora of opinion. So it's important to acknowledge the fears and concerns that families may bring up. As silly as they could sound to a researcher or a clinician, these families don't necessarily know any better than what they've heard, been told, or read on the internet. So it's important to acknowledge their fears while also being well informed of the study so that we can give them clear answers and help them along in the process. Next slide. So the importance of body donation is in general we have organ donors who save lives and we have tissue

donors who are able to help someone when they need a physical appearance, skin grafts, and transplants, and all of this stuff is incredible, but there is also so much to be learned and done from a decedent and this is why we do autopsies for research. Next slide.

Autopsy research specifically for RECOVER can be done with or without organ donations. So if someone had their desire to use themselves to save another life and they have healthy organs that the family wishes to donate, that can still be done on top of the research. So we wanted to just make that known that the research is not undermining saving the living, it just can be done in conjunction with organ donation. This research study will help scientists, clinicians, and families learn more about long COVID, helps us understand where the virus lives in the body and the types of damage it's doing. It will give families answers about why their loved one died and how that knowledge may help others and help the medical community progress and help treatments change for people who are currently living with long COVID symptoms. It can help identify the treatments and potential cures for some of the after effects. And it does more often than not provide families with a sense of meaning and purpose and that they were able to help people who are living through donating their loved one to the tissue pathology study. Next slide.

So I have found during my time as a site coordinator and speaking with families that they appreciate our ability to be precise with our answers, however difficult we assume it is for them to hear. These are not conversations people dream about having and they're not necessarily conversations that people ever expected to have. And we also just need to remember that they still deserve the same correct answers, and information, and precise responses even while they're grieving. So it's important to be kind and compassionate. Expressing our condolences to families and loved ones does go a long way by recognizing that this is a difficult time for them and that as much as we want to progress with our research, we are respecting their time to grieve. We need to be patient allowing families to process at their own speed, which is why I so heavily push for these conversations to occur earlier on, so families have a chance to process, have a chance to think about it.

We give families multiple opportunities to ask questions. As a site coordinator and a patient liaison, I'm available. I email families, call families, text families. I let them know this is a work phone, so whatever you share with me here is work related. It's not my personal cell that anybody can check and a lot of them have actually ended up calling me later to thank me. I've been invited to memorial services and it's just very comforting for myself to know that although we're doing a difficult job, it is in the grand scheme of things helping people. And another way that we wish to help families is to provide them with support materials for autopsy. And I like to always remind clinicians, remind families, that autopsy sounds scary, but autopsy provides us with answers that we might not have had without one. And answers can help provide us with closure to help us move on and to help us in our grieving process. Next slide.

RECOVER wants to be able to help all of these clinicians and help all of these families and we have created handouts and brochures that outline the study and they hit all of our key information points. So this was

something that I really loved being a part of early on in the RECOVER Consortium and being created is that I was able to help write these documents and help make sure that the information was being put into language that families would be able to digest a little easier than if we were discussing this with another clinician or another researcher. Posting these seminars to recoverCOVID.org allows the information to be on the internet, allows families to go home and sit with their thoughts and be able to look up what they need to look up and having it right there as opposed to googling things and seeing horror stories that happened years ago that science has since moved forward with.

And having this information available to physically put into family's hands and to digest on their own time is key in fostering the long lasting trust between the public and the medical community that our nation really needs. And so for my last slide, I just wanted to show a few of the things that we've come up with to better assist families and clinicians. We do have a letter to the healthcare network that describes the study and how they can help us enroll decedents. We have the clinician handouts to give to families to say, this is the study, this is long COVID, this is what it might be doing to your loved one, and this is why we want to research it.

And then we also have a brochure that we made that families can take home and go over and discuss. And everything has a QR code that leads to the website and we wanted to make sure that we were being as transparent as possible so families know they can trust us when we say this is our study and this is what we're doing and that we're all here for the greater picture of helping people to not suffer from COVID in the future. Thank you.

Jeran Stratford

Great. Thank you, Stephanie. And now we'll transition over to Dr. Lauren Decker.

Dr. Lauren Decker

Thank you everyone for coming and thank you, Stephanie, for that presentation. I think that consenting patients is probably the hardest part of our job, so thank you for what you do. I am here to discuss what it means to be a forensic pathologist involved in this type of research study. I, like many forensic pathologists across the country, am not a researcher at heart that beings that my talk is not going to be research heavy. I think Dr. Stone will do a great job of delving into that in a little bit. Being part of the RECOVER effort sort of fell into my lap, but I'm extremely proud of how well my team has been able to contribute to these efforts so far. So I'm going to talk a little bit about the differences between our group, the medical examiner's office, and other autopsy groups, the pros and cons of being a medical examiner's office while trying to participate in this research, and then discuss the thing that being a forensic pathologist is all about, determining cause and manner of death. Next slide.

So I'm not going to belabor this. Stephanie did a really fantastic job describing the study, but basically anybody who is over the age of 18 and who has had COVID more than 15 days ago and then has died within 24

hours can potentially be enrolled in this study. Next slide. So the overall study goals are 700 decedents total and you can see the breakdown between the subacute cases between 15 and 30 days after symptoms, and then people that have PASC and people who do not have PASC. Our specific cycle is 226 decedents or about a third of the overall numbers. And this seems like a really high number, but I'll explain why our goals end up being the highest of all the autopsy groups. Next slide.

So I work in a medical examiner's office, which is actually pretty unique among medical examiner's offices in the country for a couple of reasons that make us perfectly adapted to be part of the RECOVER study. So first and foremost we're affiliated with the university, which is unusual, and we have the benefit of support and the networking of academic pathology practice and all of the doctors in a university setting. Second, we are a statewide office and that means that we receive death reports from all over the state and even parts of Arizona. Most of New Mexico is rural and that falls within the special population's category that we're really trying to get a good representation from in this study. The third reason we're unique is that our office is BSL-3 capable, which means that we have the negative pressure autopsy suite. We didn't have to change our practices at all during the COVID pandemic and doing autopsies on COVID cases is not an issue at all.

And then lastly, we have our own in-house imaging. So we have a CT scanner and we use it pretty extensively. As a medical examiner's office, our decedent demographic is a lot different than your average hospital based death, although these will often get reported to us as well. So we do have access to those types of cases. Our jurisdiction covers sudden, unnatural, and unexpected deaths. In the year 2021, over 10,000 cases were reported to our office and in any given day we have between 10 and 30 cases that are reported. This includes both jurisdictional cases and non-jurisdictional cases and those are the cases that we screen each day. Next slide.

So every group has their own unique screening process and I'm sure everyone's is just as complicated, but I'll go through ours briefly. Our daily routine for case screening is a group effort for sure. Myself or my co-PI go through a generated report of all the cases reported to our office starting at 6:00 in the morning. We rule out all the cases that have postmortem intervals over 24 hours, cases that are potential homicides, cases with extensive trauma, and we also have to eliminate any cases that have evidence of decomposition. So remember that we are getting cases that don't die in the hospital always. So these are people dying at home. The time of death isn't always exact. So we use the presence of decomposition to help us with that 24 hour interval. We then call the New Mexico Department of Health. We have a great relationship with them and they have specifically allocated personnel to work with us starting at 6:30 in the morning. We call them with our list of decedents that we have left and they go through their COVID testing database.

They will look for any positive results in the past two and a half years and then we have a list of cases that have confirmed positive COVID in the past. Following that, our awesome research coordinator is able to get in contact with any of the next of kin of those positive cases and try to obtain consent, which as Stephanie alluded to, can be very difficult. If consent is obtained, then we transport the decedent to the office if we need to, if they're

not already in house. We're slowly working with our office investigators to encourage them to ask more questions about COVID history and that is gaining some traction. They're doing a much better job and also asking questions about PASC symptoms or long COVID symptoms. We realize that a lot of people have never had an official test and so it can be definitely really difficult to find those people who have never gotten tested, who have never been to a doctor's office. UNM also has an adult long COVID clinic and an adult recovered group in this study.

And so we're working with them to help create a flow from their group to ours. The issue with that obviously is that it requires one of their patients to pass away to be then enrolled into our study. Next slide. So once the body arrives at the office, we start the autopsy and there is a lot to do for these autopsies. We collect blood, cerebral spinal fluid, stool culture, bronchial swab, and tissue from anywhere from 46 to 50 sites depending on the presence of lesional tissue and the gender of the decedent. At each site we collect one sample for formalin fixation and then two samples for flash freezing. And in my experience, these autopsies last anywhere from two to four hours. Next slide.

We are lucky enough to have several isolation suites at the Office of the Medical Investigator here in New Mexico and we have our own isolation suite that we have set up where we can have everything ready to go at a moment's notice. The setup alone takes a lot of time and effort. There's a lot of cleaning and you can see there's a lot of containers for all of the different tissues and there's a lot that goes into these autopsies. Next slide. And after the autopsy, we still obviously have a lot of work to do. All of this information needs to go into REDCap. We have to try to locate medical records when they're available. Again, being part of a medical examiner's office means that we don't always have those medical records at our fingertips like if someone had died in a hospital setting. But we do do our best to call next if kin to get a medical history. And then all of this information, and again, the autopsy findings, the autopsy report after it's written, all of it gets put into REDCap. Next slide.

A subset of the decedents, right around 50% is our goal, will consent to brain retention half. And in those cases, half of the brain is saved. It's fixed in formalin for four weeks and then it's imaged with a 3 Tesla MRI. The plan is to have these images eventually read by a neuroradiologist in order to identify any abnormalities that can then be sampled histologically. Next slide. So this protocol is still, I would say, in its infancy. We have completed two quarters at this point. In the first two quarters, our site has performed 28 RECOVER autopsies, which is roughly half of the total amount that have been done at all the sites. 18 of these cases were jurisdictional. You can see the gender breakdown and the age breakdown. Again, there are not very many of them, but we have a good range of gender and age. The ethnic breakdown is present as well. Half of the cases in our group were considered a special population and just over half consented to brain retention.

The chart at the bottom right hand shows the PASC status for the cases that we know of so far. So we have three cases which fall in that 15 to 30 day window, so subacute cases, three cases with confirmed PASC, and then seven cases without PASC. Many of our cases are still pending a review of medical records or calls to next of kin to verify whether or not PASC symptoms were present at the time of death. This is again sort of a downside to

being a medical examiner's office, we don't necessarily have those histories at our fingertips. However, we are able to catch people who may never have been to a doctor's office or a hospital. So all of those people who got COVID and stayed at home or never tested, we're still catching some of those.

Next slide. So I'm going to present our first case. This was an 82 year old woman who had a documented history of COVID in February 2022. Despite being 82 years old, she had no known medical history. She was hospitalized at the start of her COVID infection for a couple of days. And while she was in the hospital, she suffered a Type 2 or a demand ischemia myocardial infarction. She was released after recovering from that, but continued to have difficulty breathing up until she passed away on March 30th, so just over a month later. At the time of her autopsy a nasopharyngeal swab was still testing positive for SARS-CoV-2. Next slide.

As I mentioned before, our office has a CT scanner in house and we perform CTs on every decedent who comes into the building. Early in the pandemic, we were able to recognize the classic radiologic findings of COVID infection and those are shown up at the right hand, the right upper corner. And you can see some variable density of pulmonary opacifications surrounding the heart, which is in the middle. And then for comparison, I put what we would consider a normal CT image in a postmortem setting down at the bottom right hand corner. And you can see there's a lot more black in the lung fields, which is air and that would be more normal. Our case is the bigger picture on the left and you can see that it's a mix of the two. So there's some variable opacifications almost in a branched pattern and that is suggestive of pulmonary fibrosis. Next slide.

On autopsy, she was found to have severe coronary artery disease. These are sections of her coronary arteries here and they are almost completely occluded by atherosclerosis. Next slide. These are sections of the heart. You can see is some tan discoloration toward the bottom part, that's the posterior aspect of the left ventricle. And this finding is consistent with a healing myocardial infarction of the right coronary artery distribution. Next slide. Some other findings, her kidneys had very granular or irregular surfaces with areas of scarring and you can see that the right kidney is severely atrophied. All of these are signs of long term high blood pressure. Next slide.

While her lungs were grossly unremarkable under the microscope, they definitely showed a different picture. For those of you who are not pathologists on the call, this is a picture of what I would consider a relatively normal lung. We have open alveolar spaces, thin alveolar walls, and relatively little within the actual alveolar spaces themselves, in this case just a couple of macrophages. Next slide. To contrast, this is a picture of an acute COVID infection with diffuse alveolar damage. So much of the air spaces are gone at this point, taken up by that bright pink amorphous material sort of in the middle. Those are hyaline membranes and there's a lot of chronic inflammation mixed into this picture here. Looking at this histology it's not hard to understand why these patients have such a hard time breathing. Next slide. So similar to our CT findings, the lungs are kind of a mix of the two pictures while the alveolar spaces are relatively open, the septa, so the alveolar walls themselves are thickened

and fibrotic and there's still a little bit of residual chronic inflammation here. So it fits with the CT picture that we saw. Next slide.

Given the entire picture, her cause of death was determined to be atherosclerotic cardiovascular disease with a recent COVID infection as a contributing factor, which brings me to the topic of cause and manner of death determination. This can be especially difficult in these types of cases. Next slide. So to give you some background, the cause of death is the actual disease process or injury that initiates death or the cascade leading to death. Some examples are listed here. There is not a comprehensive list of causes of death to choose from. Doctors get to choose the wording that they put into the cause of death or the death certificate and that can lead to a lot of variability even within a medical examiner's office. A contributing factor is a process or processes that are known to significantly contribute to death mechanistically, but are not the underlying cause of death. So for instance, if you have heart disease, diabetes mellitus is a common contributing factor.

The manner of death pertains to the circumstances by which the death occurred. There are five options to choose from, those are listed here. In order to determine that something is the cause of death, a physician should have either a documented medical history of the disease process or physical findings on an exam that indicate that this process was the most likely cause of death. Next slide. So originally acute COVID cases were difficult mostly because the extent of acute COVID infection was still being figured out. So we saw a lot of pulmonary findings of COVID pneumonia. That was pretty easy to figure out at the beginning and we saw a lot of people develop fatal PEs that ended up having COVID with or without actual symptoms, so they just tested positive. And this finding was so prevalent that we ended up testing anybody who had a pulmonary thromboembolism regardless of history or exam findings.

We eventually figured out that cardiac manifestations of COVID was a real thing and that there were neurologic manifestations as well. However, those neurologic findings can be extremely difficult for a medical examiner's office to find. Without a clinical history, it's very likely that we miss some deaths related to these sorts of manifestations. And now that people are more and more vaccinated and the virus strains are mutating, we're seeing less frequent significant disease, especially pulmonary disease, which sort of begs the question when a person dies and test positive for COVID, did they die from it or did they die with it. Without a symptom history, it may be impossible to know with our current knowledge. So now that we are looking into PASC, we are experiencing the same sorts of problems. All of our work is to eventually better understand the breadth of PASC and the pathophysiology behind the symptoms, but if we don't know the full extent of the disease process currently, how can we contribute a death to PASC?

I think that we all feel pretty comfortable with long term respiratory problems that some individuals end up experiencing such as our first case here. But there's some discussion about whether or not those types of findings should be considered PASC or is it better characterized as the sequela of an acute infection, similar to a scar. Our work here, this study, will help us better understand this disease process so we can correctly determine

which decedents died of PASC or with PASC. Then we can start to figure out why those people were so afflicted and how we can prevent this from happening in our living patients. Next slide.

To finish up, I thought I'd share the cause and manner of deaths for the cases which have final autopsy reports. And as you can see here, there is a pretty decent variety. And then on the next slide I have the same graph, but I have highlighted six cases here that are worth noting. Three have what is currently defined as PASC symptoms and two of those deaths were deemed related to prior COVID infection and one was not. Two cases were in the subacute phase, although only one death was contributed to COVID. And another case was enrolled for a prior infection, but when we did the autopsy, we discovered that the decedent had a reinfection at the time of the exam. So in just this small subset of decedents, you can see the complexities we face as a medical community in correctly identifying PASC and assessing the role that it plays in death. Next slide. Thank you for listening and being here.

Jeran Stratford

Thank you, Lauren. We'll now turn the time over to Dr. Stone.

Dr. James Stone

Hello, my name is Jim Stone and I'd love to talk to you today about some of the autopsy findings that have been observed in patients dying from COVID-19 and some of the insights this may give us into the pathobiology of PASC. Next slide. So just for reference, the acute phase of the disease of course is when you're typically symptomatic with fever, possibly more progressive symptoms like shortness of breath, but some people will continue to have symptoms long after the acute phase for months afterwards. And these are collectively known, these symptoms are known as PASC and these can involve multiple organs throughout the body. And I'm going to try to highlight some of the autopsy findings in some of these organs, particularly in the patients that have been dying from acute phase. And these are published studies largely on patients dying in the acute phase and somewhat the extended acute phase. Next slide.

So you heard about some of the pulmonary changes from Dr. Decker and I'll reiterate a key finding. If you look in the top middle panel, you'll see the red rings, which are hyaline membranes marked with stars that indicate the presence of diffuse alveolar damage. On the top right, in some areas there'll be these spindle shaped cells indicated by the arrows that are starting to organize that. So this is actually diffuse alveolar damage that's focally organizing. In the middle row on the left there's a neutrophilic infiltrate, an infiltrate of neutrophils, indicating potential bacterial infection. The bottom left indicates more of a chronic inflammatory infiltrate. These lungs, as you heard, also have a lot of chronic inflammation. On the bottom right there's a microthrombus and the lungs in patients with acute COVID certainly have a lot of microthrombi, not atypically.

On the middle row on the right is an airway that's plugged with mucus. All of that material at the top of that panel is mucus filling an airway, which is also a common finding. In the center is an early immunohistochemical stain showing the presence of virus. Early on in the pandemic, that's what was available were immunohistochemical stains which we could use to try to see the virus within the tissue. Next slide. We were fortunate to be able to help in the development of the In-Situ Hybridization technology, which is really now the standard for looking at virus within the tissue. In the left panel you see one of those thick red hyaline membranes and on the right panel what you're seeing is all those red dots are amplified virus within the lung, primarily localizing within a hyaline membrane.

These hyaline membranes are really sites of dead lung epithelial cells and the viruses can be quite prominent in those areas. Next slide. So the COVID-19 single cell atlas of the lungs was undertaken here in Boston with contributions from all of the Harvard affiliated hospitals. One of the key findings from this study was that numerous cell types within the lungs actually contain virus, not just epithelial cells. The most predominant cell type to have virus was actually myeloid cells, inflammatory cells like monocytes and macrophages. Now this was a little skewed because a lot of the epithelial cells were wiped out and killed in some of these patients, which is probably why they're underrepresented here. In addition to inflammatory cells, notice in the right panel that numerous types of endothelial cells also contain virus in the lungs from arterial to venous endothelial cells. Next slide.

This is a study out of Mayo Clinic showing some of the more advanced changes as those hyaline membranes continue to organize. On the top right you see that that spindle cell phenomenon has really taken over most of the area and this is organizing diffuse alveolar damage. And in the bottom right this progresses to scar tissue. So this is pulmonary fibrosis and unfortunately scar tissue tends not to be reversible. So patients who develop the pulmonary fibrosis will likely have long term respiratory symptoms. But again, we're mostly talking about patients who had very severe acute phase disease, but pulmonary fibrosis certainly explains some forms of PASC in some patients. Next slide.

The virus can persist in the lungs for a long time, and this is a study, I believe it was out of Spain, showing virus within the lungs up to 108 days. And so in the right half of the boxes, all of the colored boxes indicate positive PCR signal for virus. Notice not every lobe would be positive within a given patient. Some lobes might be positive, others might be negative. It really underscores the importance of doing multiple sampling and not just single random sampling to try to understand where the virus is persisting. Next slide. The virus COVID-19 also causes many cardiac changes. There are numerous types of inflammatory changes. Small vessel thrombi are also seen in some patients, the frequency of which is really dependent on how well they're anti-coagulated, but they are definitely seen in some patients and the thrombi can be associated with microinfarcts and ischemic injury as well. Next slide.

So myocarditis is one of the features that we see in the heart. It's certainly not a common feature, it's not seen in the majority of patients by any means, but it is a feature that some patients do manifest. And this is an

example of lymphocytic myocarditis where there's an extensive infiltrate of the myocardium by lymphocytes. Next slide. This is a more typical example of the myocarditis in these patients. And again, I'm talking about patients dying from severe acute phase COVID who were largely not treated with dexamethasone. And in that population you can see these punctate areas of myocarditis as we have here with myocyte injury. The lymphocytes in these cases are typically CD4 predominant and again, it's usually multifocal and not diffuse. So you usually will not pick this up on an endomyocardial biopsy because they're usually not diffuse enough for that. Next slide.

Occasionally you will have a high grade myocarditis in these patients such as we have here where there's extensive infiltrate and not only just lymphocytes, but in this case even giant cells and eosinophils. But this is very unusual. When myocarditis is present, it's usually much more subtle than this. Next slide. A more common feature is in these patients is a diffuse inflammatory infiltrate often with a lot of macrophages as you see here, CD68 positive macrophages. Now this is not a feature specific to COVID. You can actually see this type of infiltrate even in people with bacterial sepsis for example. But this is a relatively common feature of patients with severe acute phase COVID is that they will develop inflammation within the heart, although it's really in most cases not defined as myocarditis. Next slide. There can be pericarditis in these patients sometimes this is a very CD8 predominant process composed of mostly cytotoxic T lymphocytes. And then you can see at the top of the left panel there's a reaction on the epicardial surface of the heart. So pericarditis is certainly another issue these patients experience. Next slide.

So because of the thrombi that form in the lungs, there's a pressure overload in the right ventricle of the heart in some of these patients and they will experience strain injury. And this is also responsible for some of the troponin increases that you see in these patients. On the bottom left panel at the top of that panel, the myocytes have lost their nuclei compared to the myocytes at the bottom of that panel. And when you look at the center panel on the bottom, those dead myocytes stain for compliment, compliment factor 4D indicating basically that they're dead in fixing compliment. And the top two panels you can see extensive staining of dead myocytes for C4D. So right ventricular strain injury unfortunately is an important component to the troponin in the patients with the acute phase of COVID 19. Next slide.

So a key question early in the pandemic is what was the role of virus in extra pulmonary organs? And it's still an important question that many people are working on. We knew from patients with severe acute phase disease that virus could be detected early on in the blood in the majority of cases. And also the majority of autopsy studies that were using fresh heart at autopsy, which is difficult because of obviously the infectious issues, but for those studies that were using fresh tissue and doing PCR on fresh tissue, they were identifying a virus in the myocardium. But the question was still there as to whether this virus in the heart muscle was within cells or whether it was just virions in the blood. Oh sorry, go back. If you look on the left side, packaged virions in the blood contain the positive strand, but once the virus enters the cell, the RNA is transcribed and you have both the

positive strand and the reverse strand. And PCR by itself, sometimes it can be difficult to know whether you're actually looking at virions in the blood or virus within cells itself. Next slide.

Next slide. There we go. Again, using that ISH technology, we can see infected cells, cells containing virus within the myocardium. These are mostly an interstitial cell, likely a macrophage and you see that in the bottom panels on the left and in the middle. And when we see this, that's usually associated with inflammation, whether it's full blown myocarditis as you have on the top left or lesser degrees of inflammation as you have in the top middle. Next slide. Interestingly, when we detect the virus using positive strand ISH, it does correlate with reverse strand of the virus as profiled using nanostring profiling, RNA profiling, indicating that there is replication of the virus going on within the cells of the myocardium. Notice that there is one case on the far right under the M negative group, the myocarditis negative virus negative group, that has a lot of green, but that's green in the positive strand sequences, but the very top reverse strand is completely negative.

And next slide. That basically corresponds to what you would see from packaged virions in the blood. So we need to remember when we are looking at tissues, there are two issues as to whether the virus is actually in the cells and in the tissue, or whether it's just in the blood. Next slide. Most of the cells in the heart are these interstitial cells, but just like the lung tissue, we can occasionally see virus and endothelial cells that we see in the top left. And in the bottom left, we do occasionally see virus within myocytes themselves. When we see virus associated with endothelial cells, it actually correlates with the presence of platelet microthrombi. And when we see virus in the myocytes, it actually correlates with the presence of myocarditis. So the presence of the virus in specific cell types in the heart appears to be correlated with specific pathologies correlating to those cell types. Next slide.

An important feature from the myocardial studies is that the inflammation in the heart is strongly correlated with time and from the duration of the onset of symptoms. And here we're looking out to 48 days from the onset of symptoms. This is true whether you're looking at lymphocytes or macrophages and the inflammation, this is a log scale, so it's a marked increase in the amount of inflammation. But what I want you to see now in the panel on the right is that this correlation with time really is due to the presence of virus or correlated with the presence of virus. You don't see this correlation in the absence of virus only really in the presence of the virus. Next slide. Moving on to the olfactory epithelium, which is an important tissue because of the loss of sense of smell that some patients are experiencing.

If you look at the cartoon on the left and you look at the right panel of the cartoon with the purple cells, the olfactory epithelium really has three types of cells. It has sustentacular cells, as well as olfactory neurons and these basal cells. This was a study from autopsy tissue from a group in Europe showing that the virus, which is in red, really co-localizes with the blue of the sustentacular cells, and that's in the two panels on the left. And the far right panel is showing blue staining for the olfactory neurons where the virus doesn't correlate in terms of expression. The cell type that's most severely involved in the olfactory epithelium appears to be the sustentacular

cells which can be replaced by proliferation of other cells. So this may explain why some people have a transient loss of sense of smell that then they regain.

However, it doesn't explain the long term sense of smell necessarily that other patients are experiencing. Next slide. A more recent study that came out of Hopkins just this year shows that there are more chronic changes, potentially chronic changes, within the olfactory tissue, both axonal pathology and microvascular pathology. And for both of these pathologic changes, not only were the changes more severe in the patients with COVID versus the controls, but even amongst the patients with COVID, the changes were more severe in those patients who had abnormal sense of smell compared to normal sense of smell. And unfortunately the axonal pathology may be irreversible or very slow to reverse. Next slide.

Changes in the brain have been difficult to identify and correlate amongst many studies in groups. However, changes have been identified. These are some of the types of changes that have been reported. At the top left you see a micro hemorrhage in the corpus callosum and in the bottom there's a pale area within the center of the field on the left. If you look on the right on luxol fast blue stain, that pale area is easier to see. And in the middle you can see there's macrophages within that area. This is an area of axonal injury. So micro hemorrhages and axonal injuries certainly have been shown to be present in the brain in some patients during the acute phase, not all patients, but some patients. And these potentially could explain some of the neurocognitive effects of patients who recover from COVID, but clearly more detailed clinical pathologic studies are required. Next slide.

A key question has been whether virus actually enters the brain in patients with COVID. And this was a very detailed study that came out of Arizona looking at 16 brain regions. And within each region, the group actually tried to look for five different viral genes by PCR. So a very detailed study using PCR on frozen tissue. The interesting thing is that virus was detected in about 38% of the patients. And again, these are people dying in the acute and extended phase. If you look at the left panel, that's the copy number at the bottom. So as you're moving to the right, that's higher levels of virus. Now the two small dots to the far right are lung tissue, but when you start getting to the bigger diamonds near the two lines, the one with the highest copy number is actually the olfactory bulb. So it does lend some support to the concept that the olfactory bulb may be a gateway for the virus. Certainly, there's a lot of controversy about how that happens, whether hematogenous, whether there may be more of a direct invasion around the neural processes.

But either way, the olfactory bulb seems to have the most virus in it as far as we can tell at this point in time. But certainly virus can be detected in the brain in patients with the acute phase of COVID-19. Next slide. Some people who recover from COVID find that they have new onset diabetes. And one of the questions from autopsy studies is, does the virus involve the pancreas at all? And this was a study again out of Europe showing that in fact the virus does involve the pancreas and can even involve the islets, the beta cells of the islets where insulin is generated. If you focus on the middle row I think is the clearest to see. And if you see the insulin staining on the left, the viral staining, and this is again an ISH RNA type of analysis and then the co-localization of the two

where now the insulin is in red and the virus is in green, you can see that the virus is in fact identified within the islets.

Now if you look at the right side, notice that the two COVID-19 patients have more inflammatory cells in their islets certainly than the control patients. Again, sepsis, you can also get a lot of inflammation in tissue, so it's not surprising that there's more inflammation in sepsis than controls as well. All right, next slide. So I just want to wrap up. My general feeling thinking about what autopsy studies are showing, and this is just a brief review of the highlights obviously, is that at sites of high viral load such as the lungs and olfactory mucosa, there is possibly damage, and I'm putting it in parenthesis, that's permanent during the acute phase. And this can explain some forms of PASC. Certainly the scarring of the lungs explains some forms of PASC. And I want to point out that the lung scarring is severe acute phase typically. There is a low level of extra-pulmonary organ involvement by the virus in the acute phase and extended acute phase and viral persistence in extra-pulmonary sites may help explain some forms of PASC and it certainly may be playing a role.

And this is obviously a key question for the RECOVER initiative. And I'll just point out that the identification of virus in extra-pulmonary organs is quite difficult and there are a lot of conflicting reports in the literature. But what you need to realize is that many of the reports that are not reporting virus are actually confounded by one or more of the following issues, either long postmortem interval, long fixation times using relatively low sensitivity techniques, or in some cases requiring very high levels of virus because it's really a low level of virus that's seen in extra-pulmonary organs. Okay, thank you very much.

Dr. Marie-Abele Bind

Thank you. These were really great presentations. Thank you to the panelists. So I'm the Biostatistician at the DRC, the Data Resource Core, and I'm particularly involved in the RECOVER autopsy study. My first topic I would like to start with the study population of autopsy studies. I think they consist usually of a particular population, mostly white I would say. And they may not reflect as we would hope the entire U.S. population. And I think I want to ask the panelists how to reach to other population that may sometimes be more susceptible to the virus. And as a NIH study, we want the RECOVER study to be generalizable to as many Americans as possible. So I wanted to start with this topic.

Dr. Stephanie Haasnoot

I think I can touch on that briefly. I know in New York City we have an extremely diverse population in the city itself and a lot of our autopsies for our site have been very mixed. I wouldn't say that anything was more one race over another, but I know we can't say that across the board, across the country, so that is a topic to discuss. There's a little bit of discourse between some cultures and some populations and the medical community, so we do hit a little bit of a wall there. So I think using these seminars, using the website, using handouts that are

translated to multiple languages can help us reach people who either aren't privy to this information originally or who wouldn't have thought that this was accessible for them. So I think it's something we can definitely still work on as RECOVER evolves.

Dr. Marie-Abele Bind

Great. Now, I have a more [inaudible 01:05:38] questions, so we... Thanks, Jim, to touch about a lot of systems. I was wondering if you would try to measure additional things, what would they be? And would you conduct the study a little bit differently if you had more funding and if you would conduct things a little bit differently? Yeah.

Dr. James Stone

Yeah. So the problem is at autopsy you can sample anything and everything, right?

Dr. Marie-Abele Bind

Yes.

Dr. James Stone

And everybody has a particular tissue they want sampled. And you just heard from Dr. Decker that there's about 50 sites that we're sampling, but we can't sample 500 sites. It's just we do have to try to focus and we are being open and flexible to the field and to advancements. Obviously, if there's specific sites that become obvious that we need to pay more attention to, we do have a process for amending the protocol and sampling those into the RECOVER protocol. But yeah, you're absolutely right. That's one of the key issues. It's just there's only so much that a study can afford to do and we're trying to be as comprehensive as we can with 50 sites. But obviously if you do 200 sites, you would see more, but there's just a financial restriction.

Dr. Marie-Abele Bind

Yeah, and for statistical power that would not be optimal either. Now I want to turn the discussion around viral persistence versus multiple infection. We are not really on top of things regarding to multiple infection in the autopsy study, we take the first infection, but we don't know if people had multiple infection or if they have a viral persistence. So how are you going to try to deal with this issue?

Dr. James Stone

It's a huge problem. So in fact, we often have trouble understanding how to label patients even as being active or recovered because what happens, it's not uncommon, it happens almost every week here that we have a patient who is considered to be recovered from COVID-19. They had the virus in the past at some point and yet we do the swab at autopsy and it's positive. And then I'm trying to explain to the family that unexpectedly their loved one actually was still positive at the time of death, even though none of them thought about it that way. And it is a big question as to whether that's just reactivation of the virus they already had or whether they obtained a new infection. I mean, certainly the most sophisticated way to do it is genomic analysis of the virus.

The problem is you often don't have enough RNA from the initial infection to know if it's a new virus, a different variant or not. Sometimes you can infer it if it's the virus you're seeing is a variant that wasn't around at the time of the original infection. But it's a big question and a big problem even to how to classify patients as to when a second infection has occurred and the definitions tend to be somewhat arbitrary to be honest.

Dr. Marie-Abele Bind

Yeah. Thank you. I have a final question regarding the different episode in the pandemic that we had. So we have seen that the variants are different, that people get vaccinated, and I was wondering what you can tell us about the issues that you have been thinking about regarding this different episode in the pandemic.

Dr. James Stone

Well for the histology, I'll say what a lot of people tend to forget is at the very beginning of the pandemic we didn't know really how to treat these patients at all. We weren't even giving them corticosteroids for the most part. And now all of the severely involved patients get corticosteroids. So the autopsies that you're seeing now are far different for the severe acute phase than what we were seeing in March and April 2020. Myocarditis isn't nearly as common now as it was at that time. But also with patients being vaccinated and also the administration of so many targeted antivirals, my general impression is that the disease we're now seeing at autopsy tends to be much more limited than what we were seeing early in the pandemic. So certainly a lot of the treatments are having a good effect. Unfortunately patients are still dying either from the lung problems or from some other cause, as Dr. Decker pointed out. And I'll echo what Dr. Decker said, we are seeing more and more patients in 2022 who are dying with COVID but not dying from COVID, patients who are in the acute phase.

Dr. Marie-Abele Bind

Yeah, I think that would be difficult to have an ICD code regarding death from COVID or long COVID really soon, I think. But it's an interesting issue and I think that that would come. All right, Jeran, I'm done.

Jeran Stratford

Thank you so much. So now we will transition over into the Q&A portion of the webinar. So we've had a number of people who have already submitted questions. Please feel free to drop additional questions into the Q&A and we'll see how many we can get through. Questions that we don't have a time to get to today, we will try and address in a Q&A document, which will be available following the presentation with the recordings. So one of the first questions that we would like to ask is actually for Stephanie, what do you recommend for sites to change at their institutions to develop a more robust and family centered autopsy and research outreach program?

Stephanie Haasnoot

Thank you for that question. I actually saw that pop up and I was happy to answer that one. I firmly believe that historically autopsy has been a taboo subject. Families feel that autopsy is only necessary in cases of trauma like for medical examiner cases. And what a lot of the public may not understand is the value of a hospital autopsy, that it can give the scientific community a better understanding of disease. So knowing that somebody died from a COVID infection doesn't tell us why and not knowing the why doesn't allow for the progression of treatment or their creation of medication to combat how COVID is damaging the organs. And the same can be said for heart disease and cancer, if we don't understand how we can't stop it from becoming the why.

And then so through the use of the brochures and the handouts and including autopsy as more of the natural conversation that occurs with clinicians and families as an outcome of treatment, especially in critical care units, we can better inform patients of their options and ways in which they may contribute to science after their death and kind of take away the fears that exist around the word autopsy.

Jeran Stratford

And Dr. Decker, there's kind of a follow up question that follows that same vein. Since you are connected to a university, are you able to integrate findings from these studies into the training of practitioners, specifically about understanding of post-infection conditions, helping us understand how the how kind of leads to the why? Can you speak to that?

Dr. Lauren Decker

Yes. So we are connected to university, we do not actually live at the university, so integrating with the clinicians is a little bit more difficult. Certainly whenever they have questions and an autopsy is performed, we're happy to discuss cases with them. I would really love to sit down and talk with a lot of the people in the ICU and have similar conversations that Stephanie is a kind of explaining where they're at the bedside and they're the ones doing the consent for the family or talking to them about autopsy, and really they're the ones who are the first people on scene. So I think there's a lot of training to be done in our university on that regard. But as far as our findings being transferred back to the clinicians, I'm happy to do that when we have some good ones.

Jeran Stratford

So one of the other questions that we have for all three panelists is how are the PASC autopsy team communicating with the public at large? I mean this is one opportunity, but are there others? Are opportunities whereby an individual might contribute to the knowledge necessary to end the pandemic?

Dr. James Stone

I think unfortunately for us, we're mostly doing it on an individual basis with families at the time of death is I think what's largely happening. We haven't, other than forums like this, we haven't had much opportunity to reach out directly to patient groups. But I think many members of our team would be willing to do that and that's something we've talked about. But we largely have been reaching out to families at the time of death and also to the clinicians, and hospice workers, and other people that are often affiliated with patients who are terminal to try to get the message out there. But I do think it's important for the general public to understand what's going on and that we have this opportunity to really understand the pathology of long COVID here.

Jeran Stratford

Stephanie or Dr. Decker, is there anything you'd like to add?

Stephanie Haasnoot

Yeah, I actually appreciate what Dr. Stone said that we do wish there were more avenues to discuss autopsy with the public, but it's not the most easily digestible topic for people to accept. So maybe if we did have more autopsy specific seminars or public forums that people can willingly join like this one to discuss things further or different aspects of autopsy further, I mean, I'd be willing because I think that the information is necessary to get out just to help science and the medical community progress in general.

Dr. Lauren Decker

Yeah, I agree with both of those sentiments. I think that you don't really know that you need an autopsy until it's too late as a family member. And so knowing that this is an option is a good one before somebody passes away, but a lot of people don't pay attention to autopsy studies until it's past the point where we can do something about it.

Jeran Stratford

Great. Thank you. That actually leads right into another question. As a RECOVER participant, are we able to sign up for an autopsy option so the family does not need to make that decision?

Stephanie Haasnoot

That is a very honorable choice and we would like to thank you for even coming up with that and wanting to be part of it, and it's appreciated. Unfortunately, it does fall to the laws in your location. Most of the time the legal next of kin still has to consent postmortem, so someone can be an organ donor, but if they're living next to kin declines, it's kind of out of our hands at that point. So I think that it's a good conversation to have with your family, with your partner, with your children. It's a tough one to begin, but it's a necessary conversation so that they can respect your wishes and can consent to what you would want after death.

Dr. James Stone

But I think that was perfect, I'll just add that from other research autopsy studies that are ongoing here, families tend to follow the wishes of their loved one who died. So if you make it clear that you want to be part of an autopsy project upon your passing, there's like 90% chance the family will agree to it even if they otherwise would've said no. So it does carry a lot of weight with your family even though legally Stephanie's exactly right. We have to go to the family the next of kin at the time of death for permission.

Jeran Stratford

Thank you. So another question that we have is what are your thoughts on the possible location of viral reservoirs in the body for PASC patients?

Dr. James Stone

Well, this is one of the key questions and I think there's certainly many potential sites where it could be. The virus itself doesn't appear to be neurotropic the way varicella is. So even though there's aspects of PASC that

seem similar to shingles, it's really not quite the same where the virus doesn't appear be hiding out in nerves as far as we know. But it is being identified in tissues and even parts of the virus are being identified in the blood long after the acute phase. And right now it's still a big question and not just finding where we can identify virus, but doing the appropriate studies to show it's truly a reservoir of replicating virus and just not some secondary late infection of that tissue.

Jeran Stratford

And a follow up to that, is there any evidence that the virus that is persisting out to these 108 days, does this differ by the virus strain at all?

Dr. James Stone

Well, unfortunately, the autopsy studies lag behind the strain changes. So we're mostly looking at autopsies studies now that were on early strains, mostly the initial strains and in some cases delta. But there really haven't been, to my knowledge, great autopsy studies clearly showing a strain difference. You're also confounded by the fact that the newer strains are occurring in the setting of such different treatments. So it'll always be difficult comparing to the very early strains what the impact of treatment is versus the strain itself. But that's definitely a good question that we'll have to keep our eyes on.

Jeran Stratford

So another question that we have is addressed to Dr. Decker. What has been the most surprising findings you have discovered since starting the RECOVER study?

Dr. Lauren Decker

I think for me it's been the fact that so many people still have virus at time of autopsy. Including what we normally do for an autopsy study, we do an in house nasal pharyngeal swab on everyone and more than I would've expected still come back positive, which just speaks to what Dr. Stone is saying. And just trying to wrap my mind around when is it acute, when is it persistent virus, that's a difficult topic, but that has definitely been the most surprising finding for me.

Jeran Stratford

So the other question that we have coming in is, is there any plans to establish a control group? I understand in pathology studies such design is difficult and challenging, but it would be interesting to establish a collection of

control tissues acquired during the same period.

Dr. James Stone

Well, unfortunately, because of the financial constraints that we talked about, we don't have noninfected control patients. The control here is really acute phase that we're comparing to the PASC and I suppose another control are the recovered no PASC patients. What we will use for controls though are the tissue that many of the sites have for especially pre-pandemic cases and cases that we feel truly are negative cases. It's actually getting harder and harder to be 100% sure a patient never had COVID at this point in time. But we'll use existing biorepositories and tissues for the controls, but the RECOVER cohort itself does not have a no virus control group.

Jeran Stratford

Great, thanks. And I think we have time for just one last question here where it says, many impacts on women have impacts on menstruation and menopause like symptoms. Has any research sampling on women's reproductive organs taken place?

Dr. Lauren Decker

We do sample ovaries and fallopian tubes on all of the women who still have them that are enrolled. And then I think there was a similar question in the chat about dorsal root ganglia, and we do take that sample as a standard as well.

Jeran Stratford

Great. Thank you so much, Dr. Decker. So we'd like to thank all of those who have presented today and we'd also like to thank our audience for attending the seminar and engaging with us through the Q&A. As a reminder, a recording of today's seminar will be available on recoverCOVID.org within a few weeks. We will also be posting a Q&A document that has responses to the questions that we received today but weren't able to get to. And then Shane, can you pull up the... Yes. Okay. So this slide represents the topics for our future sessions. R3 seminars are usually held on the second and fourth Tuesday of the month from 12:00 to 1:30 eastern time. However, we do want to note that in November and December this will be reduced and we will only be having one seminar scheduled each month due to the end of the year holidays.

We have some very exciting topics coming up and we hope to see you at these future sessions. In addition, the 2023 seminar series is currently under development and we look forward to continuing to share these webinars with you and the emerging science around PASC throughout the upcoming year. And with that, we thank you and hope that you all have a great day. And here is a final seminar, or sorry, a survey that we encourage

you to take to help us gain feedback so that we can improve these seminars as we move forward into the new year. So thank you.

Webinar Slides

To request a copy of the R3 Webinar slides, please email RECOVER_ACC@rti.org.

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