

Responses to Participants' Questions

The overarching goal of the R3 Seminars is to catalyze a shared understanding of the research being conducted by the scientific stakeholder community within the RECOVER Consortium. The R3 Seminars and the Q&As typically feature highly scientific material intended for researchers and clinicians. For other audiences interested in these topics, a link to the National Library of Medicine's MedlinePlus medical dictionary is provided at the end of the Q&As as a resource to help in understanding the scientific terminology.

This document provides responses* to questions raised by webinar participants related to the following presentations at the R3 Seminar *SARS-COV-2: Findings from Autopsy Studies* held on September 27, 2022:

- **Presentation 1: *The Importance of the Autopsy Conversation***
Stephanie Haasnoot
- **Presentation 2: *A Medical Examiner's Perspective on RECOVER Autopsies***
Lauren Decker, MD
- **Presentation 3: *Autopsy Findings in COVID-19 Deaths: Insights into the Pathobiology of Post-Acute Sequelae of SARS-CoV-2 Infection***
James Stone, MD, PhD
- **Discussant: Marie-Abele Bind, PhD**

*Responses may have been edited for clarity.

All Presenters: Questions and Responses

Q. What does Ms. Haasnoot recommend for sites to change at their institutions to develop a more robust and family-centered autopsy and research outreach program?

Response:

Ms. Haasnoot: I firmly believe that historically autopsy has been a taboo subject. Families feel that autopsy is only necessary in cases of trauma, such as for medical examiner cases. 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 the creation of medication to combat how COVID is damaging the organs.

Through the use of [informational] brochures and 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 take away the fears that exist around the word *autopsy*.

Q. Are you able to integrate the findings from these studies into the training of practitioners, specifically regarding the understanding of post-infection conditions?

Response:

Dr. Decker: Yes. We're connected to the university, although we don't 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 to those that Ms. Haasnoot has explained, where they're at the bedside and they're the ones doing the consent for the family or talking to them about autopsy, and they're the ones who are the first people on scene. I think there's a lot of training to be done in our university in 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.

Q. How is the PASC autopsy team communicating with the public at large ... about an opportunity whereby an individual might contribute to the knowledge necessary to end the pandemic?

Responses:

Dr. Stone: I think unfortunately for us, we're mostly doing it on an individual basis with families at the time of death. 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 to clinicians, hospice workers, and other people that are often affiliated with patients who are terminal to try to get the message out there. I do think it's important for the public to understand what's going on and that we have this opportunity to really understand the pathology of Long COVID.

Ms. Haasnoot: Yes, I 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 like this one that people can willingly join to discuss [the] different aspects of autopsy, I would be willing [to participate] because I think that the information is necessary to get out just to help science and the medical community progress in general.

Dr. Decker: Yes, I agree with both of those sentiments. I think that as a family member you don't really know that you need an autopsy until it's too late. 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.

Q. As a RECOVER participant, are we able to sign up for an autopsy option so the family does not need to make that decision?

Responses:

Ms. Haasnoot: That's 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 needs to consent postmortem, so someone can be an organ donor, but if their living next of kin declines, it's out of our hands at that point. I think that it's a good conversation to have with your family, your partner, or 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. Stone: I think that was [a]perfect [response] and 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. If you make it clear that you want to be part of an autopsy project upon your passing, there's a 90% chance the family will agree to it even if they otherwise would've said no. It does carry a lot of weight with your family even though legally Ms. Haasnoot is exactly right. We have to go to the family, the next of kin, at the time of death for permission.

Q. What thoughts are there on possible location of viral reservoirs in the body for PASC patients?

Response:

Dr. Stone: 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 (tending to attack or affect the nervous system preferentially) in the way [the varicella-zoster virus] is. So even though there are aspects of PASC that seem similar to [the] shingles [virus], it's really not quite the same. The SARS-CoV-2 virus doesn't appear to hide out in nerves as far as we know. But it's 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 it's not just about finding where we can identify virus, but also doing the appropriate studies to show it's truly a reservoir of replicating virus and not just a secondary late infection of that tissue.

Q. Is there any evidence that the virus that's persisting out to these 108 days differs by the virus strain at all?

Response:

Dr. Stone: Unfortunately, the autopsy studies are lagging behind the strain changes. We're mostly looking at autopsy studies now that were on early strains; mostly the initial strains and in some cases Delta. But there haven't been, to my knowledge, great autopsy studies clearly showing a strain difference. We're also confounded by the fact that the newer strains are occurring in the setting of such different treatments and vaccination. So, it will always be difficult to compare the very early strains with what the impact of treatment is as compared with the strain itself. But that's a good question that we'll have to keep our eyes on.

Q. What have been the most surprising findings that Dr. Decker has discovered since starting the RECOVER study?

Response:

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

Q. Is there any plan 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.

Response:

Dr. Stone: Unfortunately, because of the financial constraints that we talked about, we don't have noninfected control patients. The control here is the acute phase that we're comparing with PASC and I suppose another

control group is the recovered no PASC patients. What we'll use for noninfected controls though are the tissue that many of the sites have, especially for prepandemic cases and cases that we feel truly are negative cases. It's getting harder and harder to be 100% sure a patient never had COVID at this point in time. We'll use existing biorepositories and tissues for the noninfected controls, but the RECOVER cohort itself doesn't have a no virus control group.

Q. Many impacts on women have impacts on menstruation and menopause-like symptoms. Has any research or sampling of women's reproductive organs taken place?

Response:

Dr. Decker: We do sample ovaries and fallopian tubes on all the women who still have them that are enrolled.

Q. Are you examining the dorsal root ganglia of PASC decedents for disease/inflammation?

Response:

Dr. Decker: We do take that sample as a standard as well.

Q. American Indian and Alaska Native (AI/AN) populations have been disproportionately affected by COVID-19 and they also experience a high prevalence of conditions that put them at a higher risk of severe disease from COVID-19. What do we know about the prevalence of PASC among AI/AN populations? Are the RECOVER autopsy cohorts working with Tribal Nations and/or tribal, Indian Health Service, and Urban Indian clinics to include AI/AN decedents in autopsy studies?

Response:

Dr. Stone: The AI/AN populations are affected by PASC. The RECOVER autopsy cohort is dedicated to recruiting a diverse study population and welcomes opportunities to engage community leaders representing groups traditionally underrepresented in biomedical research.

Q. Are there any protective therapies for preventing/limiting entry of COVID viral infection into the olfactory bulb?

Response:

Dr. Stone: In my experience, vaccination is associated with less viral involvement of the tissues at autopsy in general. I'm not aware of studies that have specifically examined the olfactory bulb in this regard.

Q. Dr. Stone, after showing a lot of virus in the endothelium, microthrombi as a result of endothelial injury, do you agree with Dr. Peter Libby, of Harvard Medical School, that COVID-19 is actually an endothelial disease?

Response:

Dr. Stone: We should clarify that we only see a lot of virus in the endothelium in the lungs. Virus within endothelial cells in other organs, such as the heart, is relatively sparse. Microthrombi in COVID-19 derive from multiple causes including endothelial injury and activation in some patients. My opinion is, as with many other diseases, that the endothelium plays important roles in COVID-19 along with other cell types.

Q. How do you determine if someone has died with COVID [as compared with] from COVID, especially with PASC?

Response:

Dr. Decker: To determine whether a person has died from COVID/PASC, you need to determine a link between the infection and death. Sometimes, this is based on history and physical symptoms and sometimes it's from examination findings, such as diffuse alveolar damage. If these things are absent, we cannot establish that link and cannot say that COVID/PASC was contributory. This is why knowing the full extent of the disease processes of COVID and PASC are so important. If we don't know that it can cause disease, we can't adequately find it.

Q. Could RECOVER represent an opportunity for autopsy public health and infectious disease “ambassadors” to spread the word about the importance of scientific discovery throughout the life course?

Responses:

Dr. Stone: The COVID-19 pandemic has illustrated the importance of autopsies in human health, and autopsy pathologists are speaking to the public much more frequently than in years past, largely through interviews, news articles, and press releases. The RECOVER Initiative recognizes the value of autopsies and is offering an additional venue for autopsy pathologists to highlight the value of autopsies in scientific discovery, particularly relating to novel disorders of unclear etiology such as PASC.

Ms. Haasnoot: I believe RECOVER is offering to the public something that hasn't been offered routinely by research in the past, and that is transparency. We're not hiding what we're doing and what we're studying. There is a public website, newsletters, patient brochures, family handouts, and seminars that allow the public to have information available and easily accessible to them. I think this is a massive opportunity for our site coordinators, essentially the “ambassadors” of the project, to engage with community-focused groups in their geographic areas and redefine autopsy to reflect our literature. The classic purpose of an autopsy has vastly changed with scientific advancement, and we as the public have more to gain through tissue pathology studies than in the past. Many families consent to research-based autopsies as a way to find closure for themselves or as a way to honor their loved one. Individuals who wished to be organ donors but are not eligible can still save lives through participation in tissue pathology studies and helping to further advance medicine and clinical treatment for diseases like SARS-CoV-2. If we can continue to spread the word about RECOVER, we can in essence change the conversation that surrounds autopsy for research projects that currently exist and those that are yet to come.

Q. Are you testing for reactivated VZV (varicella-zoster virus, also known as shingles) in the nerves in PASC patients?

Response:

Dr. Decker: Specific research questions are in the process of being approved and will begin soon. The autopsy portion is solely for collecting data and tissue.

Q. How do findings from these studies compare with [studies] from the UK Biobank?

Response:

Dr. Stone: The findings [shared] in this presentation represent the findings from across the world and not one specific biobank. The results from different autopsy tissue biobanks are often related to the quality of the tissue in those biobanks. The quality of the tissue in various biobanks can vary greatly depending on variables such as

postmortem interval, duration of formalin fixation, and availability of fresh frozen tissue. The biobank resulting from the RECOVER autopsy cohort will be high-quality tissue relative to autopsy biobanks.

Q. [I'm] eager to know if there are opportunities for a multiplexed assay of PASC biomarkers to be used in profiling SARS-CoV-2 patients.

Response:

Dr. Stone: Hopefully, the RECOVER Initiative will help clarify a set of PASC biomarkers that can be utilized in this fashion.

Q. Are studies coming or samples to be analyzed that could benefit from detection of autoantigens, viral proteins, etc.?

Response:

Dr. Decker: Specific research questions are in the process of being approved and will begin soon. The autopsy portion is solely for collecting data and tissue.

Q. [Can you] share information on novel nuclear magnetic resonance (NMR) Long COVID and the secondary disease test called PhenoRisk PACS?

Response:

Dr. Stone: The RECOVER autopsy cohort does include NMR evaluation of ex-vivo hemi-brains. The RECOVER autopsy cohort does not utilize the PhenoRisk PACS test.

Q. Autoimmune antibodies are hard to see in an autopsy. I believe that is what's causing the POTS (postural orthostatic tachycardia syndrome), etc. What specific tests should we ask western medical doctors for?

Response:

Dr. Stone: The RECOVER autopsy cohort does include acquisition of serum, which can be used for detection of autoantibodies.

Q. How do autopsy results inform how best to treat various cognitive issues associated with Long COVID?

Response:

Dr. Decker: Specific research questions are in the process of being approved and will begin soon. The autopsy portion is solely for collecting data and tissue.

Q. What evidence has been found regarding brain bleeds, their locations— such as the brain stem—and or evidence of active COVID/spiked protein in brain?

Response:

Dr. Decker: Specific research questions are in the process of being approved and will begin soon. The autopsy portion is solely for collecting data and tissue.

Q. We were pleased to see sympathetic ganglia sampling added to the protocol at our suggestion giving the very high rate (67%) of dysautonomia in Long COVID patients, and prior autopsy studies identifying ganglia pathology in POTS and ME/CFS (myalgic encephalomyelitis/chronic fatigue syndrome). Can you update us on whether any sympathetic ganglia have been obtained from acute or Long COVID autopsies and if there are any histopathology or other findings on these specimens yet?

Response:

Dr. Decker: Sympathetic ganglia are being collected. There are no findings to date.

Q. How long until diagnostics for ME (myalgic encephalomyelitis)?

Response:

Dr. Stone: It's difficult to predict the timing of the results from medical research. The RECOVER autopsy cohort is scheduled to be enrolling for another 3.5 years. Analysis of tissues will require some time after that. This will need to be followed by clinical validation studies.

Webinar Slides

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

To Learn More

- Information about RECOVER research and to volunteer for studies: <https://recovercovid.org/research> 
- Frequently Asked Questions about RECOVER and PASC: <https://recovercovid.org/faqs> 
- CDC information: Information for the general public and for healthcare providers about post-Covid conditions: <https://www.cdc.gov/coronavirus/2019-ncov/long-term-effects/>
- For medical/scientific terminology: <https://medlineplus.gov/healthtopics.html>