Dr. Sydney Stein
Behind the Mask
September 24, 2021

Barr: Good morning. Today is September 24, 2021. My name is Gabrielle Barr. I'm the archivist at the Office of NIH History Museum. Today I have the pleasure speaking with Dr. Sydney Stein. Dr. Stein is a postdoctoral research fellow in the Emerging Pathogens Section in the Critical Care Medicine Department at the NIH Clinical Center (CC). She's also part of the Laboratory of Immunoregulation in the Division of Intramural Research at the National Institute of Allergy and Infectious Diseases (NIAID). Today, she's going to be speaking about some of her COVID-19 recent experiences. Thank you very much for being with me.

Stein: Thanks for having me, Gabrielle.

Barr: Absolutely. One of your studies was looking at how the virus could be transmitted from the mother to a fetus, which is really interesting given that COVID-19 is a respiratory virus. What made you and others think that the virus could possibly be transmitted in this way?

Stein: There were a few things that we took into consideration, the first of which, at the time that this particular case occurred, we knew that SARS CoV-2 was causing body-wide symptoms. That was very strange for something that's just a respiratory virus. While there wasn't definitive proof, we highly suspected that there must be a phase where the virus was leaving the respiratory tract and traveling to the rest of the body and probably via the bloodstream. This would then set up the possibility that if a mother who was pregnant and had acute infection, she might be able to pass the virus to the infant through the placenta. In this particular case when we were contacted in regards to wanting to collaborate and see if this had happened, the hospital and the clinicians had taken extreme efforts to prevent contamination from the mother to the infant once the infant was born. It was highly unlikely that the infant could have become infected via the respiratory system. Given the symptoms that the mother presented with, we thought there was a chance that it could have come through the blood system.

Barr: Yeah. Can you talk more about that particular case? It was so interesting, the baby was born after just 34 weeks, and it was seemingly kept distant [from the virus], but then tested positive after 49 hours of life.

Stein: Yes. The mother presented to the hospital and at the time, she was having some cramping and some vaginal bleeding. When they were running her bloodwork, there were some changes that were consistent with possible COVID-19 infection. She had also reported that she had a cough for about a week. They did go ahead and do a nasal swab on her, and she was positive for SARS-CoV-2. At that point, they were not sure if what was happening with her pregnancy was related, but they knew that she was infected and wanted to prevent any transfer of infection to the infant. They needed to move forward pretty quickly with a C section.

Barr: Yeah. Can you talk about how you and others went about analyzing the cord, the blood in the cord, and the placenta tissue?

Stein: Yes. Thankfully, the caregivers at the hospital were in contact with their laboratory staff. As soon as they knew that the infant had tested positive for COVID-19, they made sure to save any remaining samples that were present on the infant. Although we didn't get quite as many samples as we would hope if this were a proactive thing we were looking at, we were lucky enough to get cord blood that had been frozen. That is very good for trying to detect nucleic acids, which is what we were looking for with my assay. Then unfortunately, the placenta

while was saved, it sat in formalin for about two weeks, which can degrade nucleic acid. With the help of the pathologists and the National Cancer Institute, they were able to look at the placenta with H&E staining [the combination of two dyes, haematoxylin and eosin] to look at the pathology. Then they did try to do some staining to look for virus and were unable in the tissue that was saved in formalin. We also did extractions from the infant cord blood and then some urine samples from early in life. I ran those via PCR [polymerase chain reaction] to look for the SARS-CoV-2 nucleic acids. We weren't able to find it in the placenta. There are a couple of reasons why that might be, but we did end up finding it in the cord blood and in one of the early urine samples, which was very exciting.

Barr: That is really interesting. So have there been other cases like this that have shed any more light on this mode of transmission?

Stein: There have been some other cases which have come very close to what we've been doing. I'm not aware at this moment of another that has found it specifically in cord blood. But there are multiple publications now where they have found the nucleic acid within the placenta, which is something we were not able to do. Interestingly, we've been looking at this recently to find if there are any reports of whether or not they have found the virus within tissues of pregnancies that have been lost. At the moment, it looks like there's not. This is something that we're also actively interested in pursuing to see in cases where the pregnancy was unfortunately lost when the mother was infected with COVID-19, if somehow the virus transferred to the fetus at that point.

Barr: Yeah, that's really interesting. Are you currently engaged in that research right now and can you talk a little bit about it?

Stein: We are engaged in the research. Unfortunately, it's not yet to publication so I cannot say what we found. But we have had multiple people from this area and other areas that have contacted us because of this publication, to see if we'd be interested in collaborating. We are currently analyzing several different tissues from unfortunate cases of pregnancy losses. We're hoping to publish those results soon.

Barr: Yeah, definitely. Another study that you've been involved in is Dr. Daniel Chertow's autopsy study. Will you speak about the premise of this study and what your contribution to the team was? Also, I hear it was quite an experience. What was the experience like for you working on the team?

Stein: Absolutely. This was a huge team effort. We collaborated very early during the pandemic. Our first autopsy was at the beginning of April 2020 with the National Cancer Institute [and with] all of the Pathology Department. I think that I came to know every single pathologist and pathology resident over the year that we were doing this up until March of 2021, and we had multiple volunteers from other institutes that came to help our small but mighty team during tissue procurement, but it was a very intentional way for us to investigate the pathogenesis of SARS-CoV-2 and what it's doing, particularly in fatal cases.

Now, that said, we were not limited to cases who died very early in infection. In fact, we really wanted to know what is happening in people who happened to die many, many months after having been infected, whether it was directly from consequences of having the virus or other reasons. We do have several patients that had complications following a transplant that either was related to having COVID-19 or wasn't. And then we had another patient that unfortunately had a sepsis situation happen from an infection and passed away. So not a direct link to having COVID-19.

This was our goal, what is happening over time? And can we track what the virus is doing in the body? Where is it dispersed? How long does it stay within those organs and at what level? Because we definitely thought that

that would tell us something if it was there. I'm happy to say that we have quite a diverse cohort, 44 patients ranging in age from six [years old], which was a very hard case, all the way to 91 years old. So we have a very good distribution. We've seen what it's doing in patients that had many comorbidities. They had lots of other illnesses and health issues prior to having COVID-19. And a couple patients that seemingly didn't have anything wrong with them got COVID-19 and unfortunately passed away. So it's been a very good way for us to actively look into what is happening there. That has been my lead in this effort—helping all of these autopsies to happen, making sure all of the tissues were procured appropriately, in many different ways, so that we could do almost any kind of downstream analysis that we wanted to on tissue preserved in an ideal way. As we talked about before, sometimes it can be really hard to run certain assays, or impossible, if you don't have a tissue preserved in the correct way.

Barr: Can you tell us a little bit about how you go about doing that?

Stein: Yes. So when we are in autopsy, the pathologists are actually performing [them]. They're at the table side with the patient, collecting the tissues. They would then pass us a portion of each organ. We were very comprehensive. At the beginning, we were collecting everything that we could other than the brain tissue, because we hadn't yet figured out a way to do that safely without generating aerosols. By the end, we did have 11 patients, [from whom] we were able to collect that. But we have every major organ of the body, multiple sites of lymph nodes, lungs. In some patients, we were able to collect upwards of 60 sample types, which was very exciting for us to be able to take such a wide look at where the virus might be and what it might be doing, and also potential immune responses that were happening at those sites.

Once we would receive the tissue, we would then preserve it in multiple ways. So one way was in a solution called RNAlater [reagent that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples] which helps preserve nucleic acids. It's ideal for downstream PCR analysis and then subsequent sequencing as well, if you found virus. We also then flash froze tissue. If you ever wanted to culture virus, you need to have just a frozen piece of tissue that was frozen very quickly so that you don't have virus degradation. Then you can thaw that, which is also something that we are doing with this study on potential samples from certain patients. We then also collected all of our samples in formalin, which is the usual preservative that you would do for any kind of downstream cutting tissues and staining on slides. Then we had another preservative, which is an ethanol-based preservative that is better for things in situ hybridization [a technique that allows the detection and localization of viral nucleic acid in tissue sections], where you're going in and staining for a particular target and then imaging that, which we are doing with this looking for SARS-CoV-2 RNA within the tissue. That just helps preserve nucleic acids a little bit better, while still inactivating the virus from infecting any user downstream.

Then we did a couple of other preparations for very specific imaging techniques. We're not quite all the way there yet with our studies, but we do have [tissue] on hand for continued downstream analysis.

Barr: How quickly from the time those tissues are taken out of the body do you need to get them into some kind of solution or flash freezing?

Stein: So we are doing it right away, actually in the autopsy suite. We have all of our tubes pre-staged for every sort of tissue type that we want. We are immediately putting the tissues, after they're being procured, into those solutions. We also have dry ice on site. We are immediately freezing and then later decontaminating everything to take out for longer storage, but one of the things that we did try to do up front of that is take patients within 24 hours after they've passed away because once you are getting past that there is a much greater risk of autolysis, which is the tissues breaking down naturally after the host has passed away.

Barr: So were you having to be on deck at all hours of the day and night?

Stein: We did some very late-night autopsies, which was not ideal. Eventually we did say we will stick to trying to do autopsies starting in the morning into early afternoon, but we won't come in at night because we don't want to put anybody at potential harm while we're using scalpels and sharp objects with infected tissue samples. But we did have a text chain going and my PI, Dr. [Daniel] Chertow, was taking calls at all hours of the night, talking with families who had just lost their loved ones, consenting [them] into the study, and then letting us know, via group text if we were having one coming up in the morning. I did put together an on-call schedule for the better part of a year so that we would have people ready every single day in case we had an autopsy. It was a very, very big lift and a lot of people put in quite a bit of time to make this study happen. It wouldn't have been possible without all of our collaborators and people who helped.

Barr: Before this study, had you been to an autopsy.?

Stein: I had never been to an autopsy. I'm actually a veterinarian, so I have been to many necropsies, which are basically the autopsy process on an animal. That has never bothered me. I had never been to a human autopsy because I actually did not enjoy going to a cadaver lab when I was in high school. But I quickly got over that with this study. So it's a lot of translational knowledge between the animal world and humans. I just had to get over the mental component of doing this work, which is very challenging.

Barr: What were some of the overall findings of the study? I know you're still analyzing the data, but are there any big observations that you all made?

Stein: Yes, so I can't speak directly to our results. But there are other publications out there, which have looked at similar aspects of what we are doing, although not quite at the breadth that we are. SARS-CoV-2 has been found in multiple organs outside of the lungs. We have been able to replicate that finding, and also [the finding] that the virus does not necessarily go away very quickly after infection. Others have also reported [that] up to a few months out, that there are still nucleic acids present within organs and inside the lung and outside. We have also found this in our work.

Barr: That's really interesting. Was that surprising that to you that the virus didn't go away as quickly as you thought it would?

Stein: Yes, it was surprising to us. In particular, a question I think everybody is trying to answer is, "Why is this happening?" Also, just clinically speaking, [for] patients that have what they're calling long COVID, who continue to experience symptoms for many months after having infection, nobody has quite figured out why is that happening and who is predisposed to this happening. That is part of the bigger question that we're also trying to answer through our particular study.

Barr: Yeah. So you're analyzing the data from the 44 autopsies that you conducted last year. Can you talk a little bit about what next steps you and your team are going to take?

Stein: So we have another collaboration that's currently happening with the National Eye Institute, where we did a more in-depth look at ocular tissues and infection into that. That paper is very close to publication. I can't really speak on the results just yet, but that is upcoming. We're also partnering with other collaborators, both at the NIH and outside of the NIH to take even deeper looks into what's happening, particularly what the immune system is doing in organs that we are finding virus in. In particular, [in] some of the patients that are farther out

from infection, what is happening there that is allowing that virus to linger? What that can tell us about what's happening out in the real world, even in patients that are not dying from their infection. They are recovered, but still experiencing symptoms.

Barr: Yeah. I had a question. Of the 44, there were some with a direct link to SARS-Co-V-2, but you said there were others who died of other things. Did you have anybody who just so happened to have COVID, like somebody who died in a car crash, and they just so happened to test positive for COVID 19 and were asymptomatic or something like that?

Stein: Yes. So we did have a few patients that came into the hospital who were not necessarily known at the time to have COVID-19 and were diagnosed, but eventually did get sick. We didn't have anybody in particular that I recall, who had like a car crash and were found incidentally [to have COVID-19]. And then we did have one patient who was a suspected case. It wasn't until after the fact that we actually found out that they had COVID-19, but they didn't present to the hospital with respiratory symptoms. So that was an interesting one. We had to do a little more legwork on the back end to prove that they had COVID-19 since there was no positive nasopharyngeal swab [test].

Barr: Yeah, that's really interesting. You've also been a part of some other studies. Could you speak briefly about your role in one that looked at high-throughput single-copy sequencing, which revealed SARS-C0V-2 spike variants coincident with mounting humoral immunity during acute COVID-19. And then you've been a part [of studies] that have looked at SARS-C0V-2 in saliva. Could you just talk about some of those?

Stein: Yes. We've been able to be a part of multiple collaborations around this study just because we have collected a vast amount of tissues from multiple patients [and] we have very good preservation methods for allowing a lot of different types of downstream analysis, one being sequencing. We definitely have had patients from our autopsy cohort. The particular paper that you mentioned is actually not from our autopsy cohort, but we also have been working on patients that were treated at NIH as part of clinical trials. The phase one and two clinical trials that were looking at remdesivir, these had patients who were staying at NIH. Since they were already enrolled in a study protocol, we were able to get respiratory and blood samples from those patients, run them via droplet digital PCR, which is the assay that I do, and then have the RNA on hand. For a few of these patients, we have had other collaborators who are interested in doing sequencing of the virus to see how it's changing over time in the same patient. Dr. Eli Boritz's lab [NIAID] did that on a few of our patients from those particular study cohorts.

And then for the [Human] Cell Atlas paper, looking at the oral mucosa, that did come directly from our autopsy patients. We were able to share RNA from oral tissues, as well as sharing tissue with Dr. Blake Warner [Stadtman Investigator and Chief of the Salivary Disorders Unit and the Sjögren's Disease Clinic, in the National Institute of Dental and Craniofacial Research] and his collaborators to look in depth at what is happening at that interface. They were able to definitively prove that yes, saliva is infected, and yes, it is replicating within salivary glands. That was also due to other people on the NIH campus, NIH's COVID-19 Testing Car Line, and things like that, that they were able to get all the samples to put that very complex piece together. It's definitely been rewarding.

Barr: It's exciting that all the samples have been used in different ways.

Stein: That's definitely the goal. It's very unfortunate that we had the study in the first place—that people were unfortunately passing away from COVID-19 infection. Our goal has always been if a family trusts us enough with their loved ones after they pass away, we would protect the integrity of that person, and do as much good for

the scientific discovery of what's happening so that we can contribute to differences in treatment, testing, and even potential public health measures around what we do day-to-day. We're working very hard to bring something good out of an unfortunate situation.

Barr: Definitely. Are there any plans to do another autopsy study looking at how different variants affect the organs?

Stein: This is definitely something that we have discussed. But at the present moment, it's not something that we are looking to jump back into. We unfortunately have a very small team and we're trying very hard to get the results from all this upfront effort out. At the moment, we're just not quite ready to take that back on. I think that there are others who are looking at this, and I would be interested to see what they find if there are vast differences between the Delta variant and previous ones.

Barr: Yeah. You said that you often, in a lot of these studies, do the PCR assay. Can you talk a little bit about what that means and what that entails?

Stein: Absolutely. I use a platform called droplet digital PCR (polymerase chain reaction). This is a little bit different than what is conventionally done. When you do something like a real-time reverse-transcription PCR that is taking a target within a sample and you are hoping to replicate it. Once replication happens above a certain threshold, there is a fluorescent target that is within the assay, and then the machine detects it, picks it up, and makes a quantification about how much is in there, if you have run a known standard curve of the target you're looking for [gesturing with her hands as she's talking].

With droplet digital PCR, what we do is that single reaction mixture that you started with [doing] the other method is then broken up into 10,000 to 20,000 droplets that are situated within oil. The PCR reaction happens within each of those droplets, and then it is read on a machine. You have the same concept if your target is present, there is a fluorescent link on there that will light up when the machine flashes it with a laser telling it that it's present. But instead of looking at it to change in real time and go above a threshold, you're reading it at the end of the reaction. It's either a yes or a no. The computer system uses Poisson distributions statistics [measures the probability of a given number of events happening in a specified time period], which is not something that I'm an expert in, but the algorithm is built in. And depending on how many yeses you had in the pool of droplets, it will tell you how many copies of your target were there. So it's very, very good for looking at rare targets. Although the more you have, the assay can get overwhelmed and you have to further dilute your sample. This is what I've been running on all of our tissue samples. We've run it on blood products. We've run it on respiratory samples, which is what the assay was originally validated to do.

Barr: Well, that's very interesting. In addition to being a scientist at NIH during the pandemic year, you have also been a person who's been living through the pandemic. So what have been some personal opportunities and challenges for you presented by COVID-19?

Stein: That is a very good question. So certainly, because I work in an emerging pathogens laboratory, in early January 2020, this was something that hit our radar before it was necessarily common knowledge on the news. It was something that I was tracking pretty intently to see if this was going to be something that might interest us. I jokingly said to my boss, I think at the end of January, beginning of February, "So are we going to start studying SARS-CoV-2 now?" although at the time it was not called that. He laughed and said, "No." But it became apparent that the virus was spreading pretty quickly.

Initially, we had actually put in a proposal to look at this in an animal model. We didn't end up pursuing that. But because the virus had spread to the United States, and that gave us a chance to actually look in humans, which historically is not something that our laboratory does, because most of the viruses that we study, like Ebola, we don't have cases here in the United States or NIH to study. And so this was definitely a chance for growth for our lab to move into a different space, although extremely challenging. Like I said, I am not super comfortable around deceased people. It was definitely a factor into wanting to go to veterinary school or medical school. And that's something that I've had to work through, which was very challenging, especially with some of the patients—there were definitely patients who were younger than I am— and so it kind of forces you to think about your own [mortality].

Barr: How did you guys deal with it emotionally? It must take a toll. How did you deal with it and remain empathetic because you don't want to be hard-hearted against the situation. But you also can't let it get you down every single day. You could be depressed.

Stein: Yeah, and I won't sugarcoat it. I definitely started speaking with a therapist about the experience of doing this work during COVID-19. It was very challenging, but it was definitely also a sense of a rewarding feeling knowing that I'm in this place at NIH and in a lab where I can actively be doing something about it to help. That was the key belief and message that drove all of us on my team to keep going even though it was really difficult, especially during the winter of 2020 when we had the largest surge. We had a lot of patients during that time that were passing away to the point where sometimes we had to turn down [cases] because we already had another case that was coming in.

So it was very challenging, but it's rewarding to be in this place. I'm not sure that there was another place that I would want to be during this pandemic. I think that the lessons that we've learned here and the experiences that I've gone through, and even the coping mechanisms, are going to serve me going forward, because this is work that I'd like to continue. I don't think that this will be the last emerging pathogen, unfortunately, we face. I am grateful to have been in a place and have the opportunity to do this work.

Barr: Oh, that's really wonderful. About how long does an autopsy take?

Stein: So these particular autopsies take in the range of two to three hours. And most of that is because we are going more slowly. There are more precautions. We're taking airborne precautions. Everybody who is present in the room has to be in a PAPR [powered air purifying respirator]. That's the personal air purifier system. And when we added on collecting central nervous system tissues, like the brain, that adds a little bit more time, setting up our device to keep that from generating a bunch of aerosols, trying to get into the tissue. So they're pretty long endeavors. But we were able to accomplish a lot within that time. I'm very grateful that the pathology team was willing to do this work with us.

Barr: Yeah, definitely. Well, is there anything else that you would like to share about your research or experiences with SARS-CoV-2?

Stein: I'm really grateful that the NIH has taken it as seriously as they have. I definitely feel like I come to a place that recognizes the importance of what's happening and is doing what they can to keep us safe. My heart goes out to all of the clinical staff on the other side of our studies who were helping refer us cases and definitely thinking of them as they're still dealing with this even though our portion has finished up on the autopsy side. So

I'm hoping that everybody out there thanks healthcare providers, when given the chance, for everything that they've done and sacrificed during this pandemic.

Barr: Well, thank you so much for all your work, and I wish you and your team continued success and of course, continued safety. It must be very nerve-racking to be around the virus so much. So thank you for putting yourself at risk for all of us.

Stein: Thank you, Gabrielle. It's very good talking to you.

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