Dr. Blake Warner

Behind the Mask

November 4, 2020

Barr: Good morning. I am Gabrielle Barr of the Office of NIH History and Stetten Museum. Today is November 4, 2020 and I have the pleasure of speaking to Dr. Blake Warner. Dr. Warner is an Assistant Clinical Investigator and he is also the Chief of the Salivary Disorders Unit and the Sjogren's Syndrome Clinic at the National Institute of Dental and Craniofacial Research (NIDCR). Thank you very much for being with us today.

Warner: Thank you for that warm introduction, Gabrielle. I appreciate it.

Barr: Absolutely. Dr. Warner, you have been very involved in a trial that you're leading, entitled Transmissibility and Viral Load of SARS-CoV-2 and Oral Secretions. Can you describe a little bit about what you are studying in this trial?

Warner: Early on in the pandemic when we had the idea that COVID-19, the disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) or coronavirus, would come to the United States, [we had] an idea that it could possibly infect oral tissues, specifically the salivary glands, as had been shown in some model systems. Looking at SARS-CoV or SARS and because of that possibility, we thought we could use saliva to test for the presence of the virus. Now we know that saliva is very useful as a diagnostic to diagnose SARS or COVID-19, but at the time in February we didn't.

We started the planning process to put together a clinical trial to test nasopharyngeal (NP) swabs— or the one that goes all the way down your nose—and compare that with spitting in a cup, a very easy test. We designed the trial around that. We didn't know how many people in the greater DMV area would get infected with COVID-19. We hoped none, but we also wanted to be prepared that it could be a lot of people. We thought if we could just be there to collect a few of them, we could make some claims about using saliva as a diagnostic.

This trial was designed to capture basically two types of people: People who had a diagnosis on the outside and wanted to get back to work at NIH whom we were going to test for the presence of virus both in their NP swabs and their saliva; and people who lived with those individuals, cohabitants, that might not have any symptoms at all but might be developing COVID-19 because, depending on a lot of different factors, some people have no symptoms whatsoever and that is a challenge to this disease. If you have people walking around who have no symptoms, they don't think they're infected, they're able to spread the disease to many other people.

When we started to think about that, we said, "What are the public health strategies that we deploy to prevent infections?" One of those is mask wearing. Mask wearing is not to prevent you from being

exposed to aerosols because at the time they're very small droplets, or aerosols, either from a sneeze or a cough or even from speaking. It's too small to get caught up in a fiber mesh. Masks are to reduce the ejection of saliva and small droplets generated by these activities. We wanted to see if we had volunteers with COVID-19 put a mask on and then give a speaking exercise and then take a mask off and do another speaking exercise or vice versa, could we show the difference in how much SARS-CoV-2 we could detect in that droplet test?

The third aspect of this study was—(we've collected saliva, we've collected NP swabs, we've asked the patients to do a mask test)—can we look at their saliva for the presence of antibodies to SARS-CoV-2 proteins? The reason that that is important is that we thought this could be used as a way to surveil if people ever had COVID-19, or if the presence of these antibodies to SARS-CoV-2 proteins could be protective, could they neutralize an infection? Because we all have the potential to [get infected], either because we touch a surface and then have our hands in our mouth as happens with children a lot, or that we drink something or inadvertently have a droplet deposit in our mouth. The presence of antibodies that neutralize that virus in the saliva, would they be protective? So, we basically designed the trial around all of those questions centered on the presence of virus in saliva. Okay. I know it's a little long-winded.

Barr: Did your study evolve from when you first started thinking about it in January/February?

Warner: It evolved slightly in that as we started to get more data and there were some emergency use authorizations for saliva to be a diagnostic, we shifted a little bit more to understanding where the virus was in saliva and the potential for saliva to be infectious. In that way we started to basically put the saliva onto cell lines and to see if that saliva and—really the virus in the saliva—would infect and kill some of those cell lines. Those results aren't quite ready yet but we're on our way.

Barr: How many people have been a part of your study so far and how many are you hoping to have?

Warner: The accrual ceiling is 90 patients. However, we have enrolled 48 patients so far and of the 48 patients we have seen about 36 in the car line. There is a third group I don't talk about; this is the recovered group and we have them come in after they're already recovered, and we can collect blood and saliva on them to look just for antibodies. But in the main groups, the ones I just talked about, there's about 38.

Barr: So, you have these different patients. Was it difficult to determine what you consider to be mildly symptomatic? How did you come up with the criteria for that list? How are you assessing those patients?

Warner: The mild to moderate symptomatic are basically patients that have any symptoms but they are able to come in and drive through the car line. If they have a severe cough or they have shortness of breath, those were the type of patients we were asking not to come through because it might not be medically safe for them present through the car line. There were a lot of unknowns in the beginning of the pandemic and we designed the study around volunteers who are not currently hospitalized. If they were able to drive or walk themselves (or a partner) through the car line. We primarily saw both asymptomatic folks with COVID-19 and folks that had very mild symptoms of COVID-19. This pattern is routinely seen in the community. Most people have relatively mild symptoms, and like we hear on the news, it's only mild until it isn't, and we don't really understand that transition quite yet.

Barr: What tools and equipment have you been using to conduct your research?

Warner: From a patient sample collection standpoint I use really simple tools. There's a nasopharyngeal swab—a long thin flexible plastic wand with a soft fuzzy tip, like a stretched out flexible plastic cotton-tipped applicator—which is how we collect the "NP" swab. We use another swab to then collect saliva from the bottom of a collection cup that we have the subject has spit in, so that we have the exact volume of NP secretions and saliva secretions to test or compare. The technique for the mask is also quite simple (Dentists are good at figuring out simple solutions). We use a standard procedure mask that you might be handed as you walk into your doctor's office. We use a very fixed volume receptacle that we have people speak into, so it kind of closes around your mouth and nose and you're able to talk like you're talking into a cup. At the bottom of the cup is a sterile collection sponge. We do the same speaking exercise both with and without a mask and compare how much saliva/or virus detected on the collection sponge. Remarkably, standard procedure masks reduce the amount of saliva detected by more than 10-fold (90% reduction).

Barr: Did you design that?

Warner: We brainstormed as a team using items that were readily available in the hospital to design the receptacle. Our criteria was that it had to be easy to assemble and very standardized across subjects. In my mind, the design was straight forward: We had a standard volume receptacle which had a collection sponge that was preloaded with some sterile saline. When the saliva droplets were ejected during speach hit that collection sponge, they would dissolve on the sponge and permit collection of the DNA and RNA afterwards.

Barr: This might be my ignorance, but do you use that sort of thing to test for other kinds of conditions and diseases or was this very much devised for COVID-19?

Warner: This is pretty much something we designed for this trial because it was in a car line COVID-19 testing facilities. We didn't have our typical apparatuses available to us because there were a lot of unknowns in March and April. Initially, as people were getting infected, we didn't quite know how infectious this would be and we didn't know how effective our PPE would be. We didn't have some of the more advanced PPE equipment. We were using a very simple N-95 mask, a face shield, and a gown. We wanted to make it as safe as possible, not just for us but for the other workers in the carline COVID-19 testing facility. In that scenario these are the tools that we had to use. In general, everything had to be quite simple in the car line.

Barr: Now in terms of one way working better than another, are they pretty much equivalent?

Warner: There are techniques that are easier for the lab to test. Such as, spitting in a cup or putting the swab in the saliva and then putting it directly in the testing media that we can then do the PCR test on. That's very easy and so that's the one we use the most. There are some other things that we do so that our research lab can also test. We use a little couple different methods to collect saliva from the subjects- for example, we can collect individual gland secretions-submandibular, parotid, secretions. In this way we can really decipher which glands are contributing the most virus to the saliva. These are questions that really remain open-ended.

Barr: That's very interesting. Have you devised any kind of techniques since you began this?

Warner: I think we may have come up with some new techniques or methods in the process of this research. We have started to test antibodies in the saliva using luciferase immunoprecipitation system which was first developed by Dr. Peter Burbelo in the National Institute of Dental and Craniofacial Research. Using that test on saliva to detect antigens to SARS-CoV-2 is novel. In fact, we did find antibodies to nucleocapsid and to spike in most patients. What we found is that patients that had the largest level of infection in their oral cavity correlated with the highest level of antibodies in their saliva which in a way is expected. It gives us a good indication that, if you have these antibodies here, that you had an oral infection and that oral infection correlates with specific symptoms like loss of taste. For the general public, loss of taste being an oral infection marker I think is kind of cool.

Barr: That is really cool. I've heard that different kinds of saliva are better than others in terms of testing. Can you talk maybe a little bit more about that? Maybe that's just a kind of one of those tales going around on the internet.

Warner: What I would say is that I don't have a lot of data that's really specific to this, but I would say that submandibular secretion specifically don't have very much, if any, SARS-CoV-2, in my hands. I can find the virus in whole unstimulated and parotid secretions. This is unpublished, but I can also find it in the sloughed epithelial cells of the oral cavity. We always shed cells from the gums, from our cheeks, from the top of our tongue, and those cells sometimes exhibit high levels of infection as well. So, when we spit in a cup, it's a combination of secretions from your cheeks, secretions from these really small glands that line the entirety of the oral cavity, and also from all of the cells that fall off of the gums. We can find virions floating around in the saliva. We can find RNA inside the cells from that, basically come out of your glands and come out of your oral mucosa. We haven't done the next experiment where we're asking which cells have the highest level of virus and how much virus is in the fluid? If we just take the fluid or just take the cells and ask which one of those, in fact, infects cells? So, these are unanswered questions. What we know is that the oral infection is very fast, meaning that we clear the infection from the oral cavity somewhat faster than the NP swab.

This is all data that's under review but I hypothesize that the clearance from the oral cavity probably indicates the times that asymptomatic folks become less infectious and it's those asymptomatic people that we never knew they had an infection. Asymptomatic COVID-19 folks are probably spreading a lot of COVID-19 out there because it can be spread potentially by saliva. I don't think that it's only symptomatic spread or asymptomatic passive activities such as breathing through your nose spreading COVID-19. Instead, I think it's an active processes like talking in public, being in close proximity, generating these potentially infectious aerosols and droplets and being exposed. This is what makes these studies challenging to do.

We don't asymptomatically test every employee every week. If everyone received their COVID-19 test every week at NIH, if we had that level of data, we would capture a couple folks that had high levels of virus and high levels of NP and saliva viral loads but have no symptoms. These are potentially the most infectious individuals. In our car line the way that we do clinical research is that patients have to consent and come through the consent process which takes a little bit of time. It's probably those times when they are basically clearing the infection.

Barr: I understand that. So, what have been some challenges you have experienced so far with your study?

Warner: I think there were challenges, and maybe there are two challenges that I want to talk about. One one hand, there were challenges with working during a pandemic. My wife and I both work on COVID-19 related projects at NIH. That is a challenge because we have two kids and the unknowns about childcare and when they are going back to school and all of this stuff. I would say I am lucky that my wife can work from home, especially during April, May, and June when I saw the lion's share of patients. We balance the childcare responsibilities, with my being here (NIH) in the morning seeing patients, her shifting her workday to the afternoons. That was a real challenge. It meant very little sleep for both of us.

If we're talking about what are the challenges with doing clinical research, I'm really lucky that we had a clinical team on hand and we had a laboratory team ready to go. We got approvals from our scientific director, Matthew Hoffman, BDS, PhD, and our clinical director, Janice Lee, DDS, MD to do the clinical study and the laboratory testing and be able to bring people back to the lab. We had high level buy-in from NIH leadership that supported us in executing the project. We had a record timeline from concept submission to IRB [Internal Review Board] approval for this clinical study. I think it ended up taking something like three weeks. This meant a lot of sleepless nights for the whole team, but it was only through team efforts like this, that we could get it done so efficiently. Ultimately, I think we were all happy to be able to work and do our part during the COVID-19 pandemic. We were able to pivot so rapidly because we had this expertise. But without all their buy-in, the Leadership, the Fellows and PAs and the Research Nurses, we wouldn't have gotten it done. I would say those are challenges, but they were fun challenges.

Barr: That's really great. Has anything surprised you so far about the research you've been conducting?

Warner: I was pleasantly surprised at how "good" the NIH community was at adopting COVID-19 social distancing and mask wearing guidelines. Because most of our subjects were NIH employees that were getting tested through the car line, I was pleasantly surprised at how few employees contacted COVID-19. I think this is a testament to NIH leadership's decision in planning to scale back operations or to send people home and how they have had a staggered approach to returning people to work. Especially, with increased flexibility with work from home. I think that I have been pleasantly surprised we didn't have a huge wave of infections. I think our infection rate is still extremely low, low enough that I amended my protocol to bring people in from outside NIH. That was a challenge; amendments were a challenge. But we got it done.

Barr: Do you plan on doing any subsequent studies from what you have found so far ?

Warner: Absolutely. I would say that an investigator could spend years just in the oral cavity investigating just SARS-CoV2. It is an incredible finding that SARS-CoV-2 infects specific cells in the oral cavity where it can replicate and establishes a model of oral infection transmission. Our next step is working with the NIH COVID-19 Autopsy Consortium lead by Stephen Hewitt, MD, Daniel Chertow, MD, and David Kleiner. Sadly, as we all know too well, people do die from COVID-19. Through these tragedies, there are opportunities to learn. We're really lucky that there are critical care medicine specialists and anatomic pathologists that perform autopsies. They had the foresight to see this as a critical opportunity to better understand the pathogenesis of disease.

I study salivary glands and not very many people do. I have collaborators that are residents and attending pathologists and I heard that COVID-19 autopsies from the community were coming to NIH. I said, "Can I please get oral tissues?" and they did. That's the beauty of working at NIH - there's lots of groups of people that really love science. So the NIH COVID-19 Autopsy Consortium was collecting

salivary glands for me from the very first COVID-19 autopsy. Looking at those tissues for the presence of virus and the specific cell types it infects and permit viral replication are critical to understanding the kinetics or the dynamics of infection in the oral cavity. More general questions we have are: if one has either or both an oral infection and/or a nasal infection, which one occurs first? Or is your disease severity different dependent on the route of exposure or the first organ infected (nasal infection or oral infection or lung infection and what is the consequence)? I think there are also research questions about what is the inflammatory response to viral infection in the salivary glands and also in the oral mucosal tissues. Secondary to that, is there any potential that this infection in the glands can actually precipitate an autoimmune disease-like state? Then this question ties back to my main research which is Sjogren's Syndrome. In fact, in the literature right now, you're starting to see some reports that COVID-19 either exacerbates or leads to autoimmune disease-like states. So that's the sort of one year, two year, and five-year projected studies that we are thinking of for this line of investigation. I'm not sure yet where this is all going to take me and how much effort we parse off into COVID-19 from our regular research ambitions. It sort of grows organically like many things in science that you chase down the interesting leads.

Barr: I have two questions that follow from what you just said. What do you think some of the practical implications will be, if you find that you know it's the virus in the mouth, do you think there are things that they'll have the public do? We already are mostly trying to wear a mask, but do you think there'll be more… I don't know protocols about what we should do.

Warner: I think there are two things that are going to happen. One: I would say it should strengthen the public's resolve to wear masks in any public environment. If you're in a grocery store, wear a mask, wash your hands, and maintain social distancing. Getting the entirety of the American public and the world to really abide by this is the only way we're really going to control coronavirus or COVID-19 until we have a widely disseminated vaccination strategy.

Barr: The second important question is, are there implications for professions that use the mouth, like dentistry? Are there infection control procedures in addition to wearing a mask or wearing goggles? Wearing an n95 can reduce the risk of transmission and maybe there is an oral rinse that basically deactivates the virus in saliva, so that healthcare workers are decreasing the risk of transmission?

Warner: I think some of those tests are being done right now by pharma and other industry groups. The effectiveness of that will remain to be seen, but I don't think it will be an either/or, not "I used mouthwash today, so I don't have to wear a mask" reasoning. It has to be: I use mouthwash and it seems to be effective. I'm going to wear a mask and we're going to reduce the risk. If we look at groups of people that do follow all these public health guidance and they keep the clusters of people they engage with small, those are the people that are least likely to be infected. There's the sentiment out there that this is a really mild infection. The truth is though, it's really mild until it's not. We don't know really well yet who will develop serious infections. In my opinion, if you're concerned about the safety of

your family, both old and very young, the very well and the very sick, then you will abide by these restrictions and public health measures to reduce the risk of transmission. That's how I hope this data is used.

Barr: The second thing is you are doing all his COVID-19 research, are you also carrying out other aspects of your job simultaneously and, if so, how are you balancing the two?

Warner: Yes, we started the clinic up again this summer and we have a reduced clinical load. I see Sjogren patients; I see other types of patients with salivary gland dysfunction. I have a really strong clinical fellow with whom I am able to spread some of that workload and then we have physician assistants and research nurses who also help with the day-to-day. In a way, it's a significant amount of extra effort but at this point we're now very able to get it all done. The sacrifice was for me in terms of sleep as I was sleeping very little. Trying to get papers written, the data processed, and the amendments in while we were in a pandemic and those are the times that are important that you do your part. Even though my part is to look in the mouth and to investigate the virus in the mouth, I still feel it is an important facet of this complex situation.

Barr: Now we are going to go into more personal questions related to the pandemic. Were you mostly working on campus, at home, or a combination of the two during the course of the pandemic and what has it been like?

Warner: I was mostly working at work. But you know scientists, we work at home too even when we are not at work. The biggest challenge for working for me was that 1. was a lack of sleep and 2. it was hard for me to lead my team. Am I leading them properly? Am I giving them what they need to succeed? I just cannot feel how people are doing when I'm not in the same room with them. That was a challenge. I would say that even on a zoom call if you ask somebody how are you doing, you don't know what's going on in their background, you don't know they might have something they can't tell you something about. Not that this is happening in our group, but that those are the kind of conversations that I have every day with my lab staff and with my clinical staff and I think it helps keep the team together. I felt a little bit detached from the team when people were not in the lab, and so, because I was able to bring a lot of my team back in, I think that we mitigated that issue. I guess concerning my leadership style, I had to continually ask myself: Am I doing the right job at the right time in the right way?

Barr: That's definitely hard. You spoke about some of the challenges. What have been some of the personal opportunities for you at this time?

Warner: Like I said. I'm really thankful that NIH is a work environment that would permit me to run with an idea and that has really been an opportunity. It's been an opportunity not just for me but for my team, to see everybody come together in this pandemic.

Barr: How many people are part of your team?

Warner: I would say for the COVID-19 study we have two research nurses that helped with this. That would be Eileen Pelayo and Carol Webb. These are the people who recruited and enrolled the subjects, no small task when it has to be all online. In the Office of the Clinical Director, Beth Briante, spearheaded getting the protocol together through IRB and through SIV. We also have the clinical director, Janice Lee, DDS, MD. She was in instrumental in getting this project operational. Early on in March, 2020, we had a short conversation about what were we going to do and how are we going to keep working? She said, "Would you be willing to see patients?" and I replied, "Yes, of course. I think SARS-CoV-2 is going to be in the saliva." She answered, "If you think this and it's worthwhile, let's do it!" So, having this level of support from your Clinical Director was instrumental. Then, having one clinical fellow, Jose Maldonado, DDS, PhD, (mentor: Jay Chiorini, PhD), was also eager to see subjects. We needed redundancy on the team as there was the potential that if one of us got sick, we'd have to have some backup. Then, I have a Physician's Assistant, Ms. Margaret Beach, she is the person that make makes the clinical operation work, from the Sjogren Syndrome Clinic all the way to working in a car line. She is my right-hand person so I was very lucky to get to work with her. For clinical testing, we worked with the Department of Laboratory Medicine and Karen Frank, MD, who did all the CDC SARS-CoV-2 testing for the entire study and managed all the samples – this was no small effort – and we are extremely grateful.

From an NIH leadership standpoint, Lawrence Tabak, DDS, PhD was central to keeping the team's pace high to get all the various departments on board with the study. I think his setting the stage to get this protocol approved, to get the Department of Laboratory involved, and to get NIAID engaged was his behind the scenes magic. We couldn't have done it on the timeline we did without that. Then in the lab we had a lot of help from Paola Perez who is my contract research scientist. I asked her early on given that we we were going get salivary glands and saliva from SARS-CoV-2 infected subjects and autopsies and, these are the types of assays we want to perform. Can we get this done? I saw her every day; she had fun developing the assays. In the the middle of the whole pandemic a friend, Kevin Byrd, DDS, PhD, University of North Carolina at Chapel Hill, called and told me they were doing the COVID-19 study and they had ten patients that had COVID-19. So, we started collaborating. I think it was only through this sort of multi-institutional collaboration - having two laboratories working on the same thing and being able to cross-validate our results that the project progressed so rapidly. It is one thing to believe your own results and sit on them for a while and come back to them for validation. It's another to say we're going to get this published in the next month. What do we need to do to prove this result? Because we had a second lab in Kevin Bird working out of the Rick Boucher lab down at the University of North Carolina at Chapel Hill. He had that ability. They were doing some of the confirmational work and some of the mucosal work to show what sites are potentially infected by SARS-CoV-2, and in the saliva to characterize that infection, so that was a lot of their work.

The other person who was instrumental in the paper we have recently submitted, currently at Nature Medicine, is Sarah Teichmann, PhD, Director of the Human Cell Atlas Project and the Cavendish Laboratory at the Welcome Sanger Institute. Both Dr. Byrd and myself had single cell RNA-sequencing data sets from different parts of the human oral cavity before the pandemic and we also had available mouse oral cavity single cell data sets. Dr. Teichemann was able to integrate these data sets to come up with a COVID-19 or a SARS-CoV-2 entry factor scorecard to pinpoint the cell types that were likely to be infected. Through this top-down approach, we were able to predict the vulnerable cell types in the oral cavity, we had the tissues that we could confirm infection, and went to the patients and we use their saliva and NP swabs— to conclusively show 1) infection in the saliva, 2) the cellular origin of virus in saliva, and 3) the infectiousness of saliva. That was really the beauty of collaborative research during the pandemic. I smile because it was fun research to do with important implications on health. It's fun to work at this breakneck pace. I think everybody who was part of the project was really on the same wavelength with that and it was only through this really tight collaboration that we were able to accomplish as quickly as we did. But I don't think the work is done, like you said.

Barr: What's your next step?

Warner: We have a couple of directions that we are going, from developing models go looking at COVID-19 pathogenesis in the salivary glands and understanding what the long-term complications of infection are. All of those are our open questions.

Barr: That's really exciting. This is a fun question. What has been your favorite movie that you have watched during the pandemic? Perhaps you have been very busy so you might not have had a lot of movies.

Warner: I didn't watch a lot of movies where I was. I would say that I did catch up on some of the series though. I watched Homeland, from start to finish. I've been watching the Great British bake off. That's a good one. Actually, my wife got me involved in that. She said I should watch this. Well, it's going to be a cooking show and I like cooking shows but they always make me hungry and so I started watching. It's actually very relaxing. I like how supportive they are of each other and it kind of gives me time to reflect on my own team and how I can continue even after this pandemic is over to maintain that camaraderie that I think we did during the pandemic. So, A Great British Bake Off and Homeland, and I watched the series Billions.

Barr: This is the last question. What would you want to share as an NIH scientist but also as a person who is living through this pandemic?

Warner: I would say that it is extremely important to trust the science and scientists working directly for the public good. Trust your public servants, especially those from NIH. The reason is that we really are dedicated to this job. We're dedicated to the American public and to providing real information, real actionable information. I feel like I have the luxury of being outside the fray. There is no “spin” in my data; the data are the data. I get to say, masks work; here’s the data. Saliva is potentially infectious; here’s the data. I hope the people who are watching this would say, take that next step, look for the real primary sources of information, look at what the scientists are trying to tell you. You know they are not being paid to tell you this; they're being paid to test hypotheses. That is an important distinction. It may be easier for intramural researchers. We can do that. We have the flexibility to pivot and to basically focus on COVID-19, to be a part of the process. I think those people are going to give you the information that you need, either through papers or sometimes through the news, when our work makes the news.

I love working here so it’s hard for me to not be biased. I feel when I wake up in the morning: When can I get to work? I’m happy and lucky that I get to work here and get happy and lucky to have dedicated staff with whom I could not do without. I owe all of this to them. Thank you to the Team. Thanks to the NIH Leadership. Even though we were in the middle of a pandemic, doing the research has been a bright spot in a very dark world.

Barr: Thank you very much for being with us today. I wish you the best in your study and in your work. Also, I hope that you and your family continue to stay safe.

Warner: Thank you so much.