**This Week in Virology**

**TWiV 1110 Clinical Update**

Host: Vincent Racaniello

Guest: Daniel Griffin

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**Vincent Racaniello:** *This Week in Virology*, the podcast about viruses, the kind that make you sick.

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**VR:** From MicrobeTV, this is *TWiV*, *This Week in Virology*, Episode 1110, recorded on May 2, 2024. I'm Vincent Racaniello, and you're listening to the podcast all about viruses. Joining me today from New York, Daniel Griffin.

**Daniel Griffin:** Hello, everyone.

**VR:** Now we have a bow tie. It's been a few weeks.

**DG:** [laughs] I think I only missed one bow tie, but I'm not sure. Yes, this is my Ebola bow tie.

**VR:** That's good. I like the color; purple.

**DG:** Yes, this is a crowd-pleaser, so people usually like this one.

**VR:** The bow tie, not the disease.

**DG:** One of my favorites. I actually have two of these. One is a little bit thicker. This is the one that I like.

**VR:** All right.

**DG:** How many men out there have two Ebola bow ties to choose from?

**VR:** You may be the only in the world.

**DG:** I'm a lucky man. [laughter] All right, let's jump right into it. I'm going to start with a Ralph Waldo Emerson quotation. As people may know, last week I was out in nature. I was out in Yosemite, which apparently, I've spent a lot of time in my life in Yosemite. When I was younger, I think I was there for about a month in the park, climbing up in the meadows, trying to climb Half Dome and just basically coming up with stories rather than successful ascents.

**VR:** Did you make it?

**DG:** It really was, we were, I think the term was sandbagged, right? We're climbing up in the meadows, Tuolumne Meadows. We're up there for a few weeks. I think I was with my buddy, Eric Johnson, and we were nicknamed the Boulder Boys because we had headed out from Boulder. They're like, "Oh, you guys, you don't want to take the trail. You see, there are 4,000 feet of these 5'9" slabs. You guys should just throw your backpacks on, solo those slabs." By the time we got done with that and a few hundred feet up the actual proper face, we were down to about a quart of water.

It was August, and we're like, " You know what? We're going to die." [laughter] Yes. Then not knowing that we could just hike to the trail, we then down climbed, 4,000 feet of 5'9" with full backpacks on. Yes, we did not actually get to the top of Half Dome that trip.

**VR:** You were young, so it was OK.

**DG:** I was immortal back then, yes. All right. "Man is what he thinks about all day long." Actually, when I read that, I was thinking, actually, that defines, a lot of people. It certainly defines a scientist, right? Scientists are always thinking about questions and puzzles, and what do we not know and what can we learn going forward?

**VR:** I think about viruses mostly all day long, so I am a virus by definition, by Ralph Waldo Emerson, right?

**DG:** Yes. Some people say you are what you eat. This is you are what you think about. As a, we'll say, clinician-scientist, I think about my patients. I think about people all day long, so I'm a people. All right. Let us start with an article. Actually, I was stopped in the hallway by just random family member yesterday. He had some questions about this article. Vincent, you sent it my way, so let's talk about the article. “Intranasal Neomycin Evokes Broad-spectrum Antiviral Immunity in the Upper Respiratory Tract,” published in *PNAS*. Let's walk through this paper to see what these researchers did, and then try to make some sense of it.

They start by taking mice and squirting neomycin into their nostrils. What is neomycin? People are probably familiar with this. It's a generic aminoglycoside antibiotic that has been shown to turn on interferon-stimulated genes. They then euthanize different mice on days 1, 3, 5, and 7 after this administration. I say different mice because you can't kill the same mouse. You've got some mice that are sprayed, and then some of them are going to be euthanized, killed on Day 1, Day 3, 5, 7. Then what they're going to do is, they're going to collect the nasal turbinate tissues.

I'm hoping they collaborate with someone else because there's a lot of rest of the mouse to look at. What are the nasal turbinates? These are, actually these areas inside the nose. Now, similar to studies squirting neomycin into the vagina and the lower respiratory mucosa, they detect upregulation of interferon-stimulated genes. So far, I think we know this, and prior work has suggested that this is dependent upon TLR3 and the downstream signaling. We can even leave a link into that study from 2013. Actually, Akiko, who's on this paper, is part of that group as well, some of the common authors there.

Then these investigators take these K18-hACE2 mice, maybe our listeners are familiar with those, and show that, much like earlier experiments with herpes simplex viruses, influenza A, Zika virus, this application of neomycin to a mucosal surface is associated with the antiviral

effect, also works for the flu in their experiments in a different mouse model. Now, this is where it gets to be a little bit, is it appears in their experiments that one must either apply this before exposure or within four hours after exposure to the virus. I'm just going to make a note there.

Then they get a bunch of people because we all know about mice and monkeys and ferrets. Now they've got some people, and they have the people self-apply this up their noses twice daily for seven days. No one gets deaf. Then they see about 44% of the folks have a five-fold or higher induction in at least two of the interferon-stimulated genes. About 19% of the folks have a good tenfold or higher induction of greater than two ISGs. They don't then go and expose those people to SARS-CoV-2. The person who stops me in the hall is like, "Doctor, should I start, squirting this stuff up my nose?"

We had a little bit of a discussion about it. No, he's like, "Maybe I can do it every time before I get on an airplane." I'm not sure that this is ready for prime time. I'm actually a little concerned, actually, that people are going to start doing what this gentleman suggests. They're going to start squirting neomycin in different places. We have a regulated immune system. It doesn't stay on all the time, so we're not really sure about the safety and the long-term issues about constantly triggering TLR3 and turning this on. I just wanted to share this. Maybe, Vincent, you and I can have a discussion about it. What are your thoughts about this?

**VR:** This is only useful as a pre-exposure prophylaxis because four hours after exposure, nobody is going to know when they got exposed, so I don't think that's terribly useful. Then you have to apply it continuously in places where you think you might be at risk. I'm concerned that you're going to select for some antimicrobial resistance by, everybody suddenly is using this, and we're going to have bacteria resistant to neomycin. I don't know that this will provide much protection in people. How do we know? You need to do a clinical trial.

I doubt anyone would pay the money for this because it's not clear that it would have such a broad effect. Here's my main thing. We have toyed with inducers of interferon-stimulated genes before. For many viruses, people have tried to make agonists, and it has not gotten far because interferon makes you really sick. That's because the interferon-stimulated genes are made, and they have a variety of effects. You remember, Daniel, when you'd treat patients for hep C with interferon in the old days, they don't like it. They feel miserable.

**DG:** We used to do that. You feel like you've got the flu for weeks and weeks, and oh, it's miserable, yes.

**VR:** I don't think this is viable. I don't know why they would even do this experiment. I don't know what the point is. The press is taking it wrong. They're saying, "Oh, antibiotics can prevent virus infections." No. No, they don't.

**DG:** Yes, that's one of the many things I worry about is, this is not an antibiotic preventing a virus. This is an antibiotic that, as a side effect, stimulates interferon-stimulated genes. Yes, and as you bring up, this is an issue. What if we lose this antibiotic? What if we keep doing this, and now, for whatever minimal effect you may or may not see, and we don't know, we have yet to actually see, is this safe? Is it effective? It was clear that some people are going to start doing this.

**VR:** Sure. If you really are interested, you'd take the active compound, and you give different amounts intranasally to mice, and see if you have a dose response, and what's the maximal dose. What's in an ointment? Neomycin comes as an ointment, right?

**DG:** Yes.

**VR:** It's not necessarily formulated for your nasal tract, so it makes no sense. This is like garage biology. It makes no sense to do that. Come on, go get the active chemical and do the experiment properly. This is in *PNAS* of all places, which doesn't make any sense either. This is not a stunning finding. As you say, much of this has been done before. I'm very unhappy with this paper.

**DG:** I'm not sure, I don't know if our listeners know much about *PNAS*, but it's one of those where there's two avenues to publication. One is, you're a member of this august group or someone is and sponsors the paper, and then doesn't necessarily require editorial peer review to get in there. It gets slotted in, or the other is the normal. You send it in and the editor looks at it and peer review, and should we publish this? Sometimes stuff can end up in here. The other I worry about too is, yes, the media loves this. This is great media stuff, but I worry about the potential negative impact.

Is this really good science that should be in *PNAS* and all through the media and people having these ideas that they're going to start taking some Neosporin ointment, and sticking it up their nose when that's really not what they studied here. They studied basically spraying this liquid. Little worries. Now that we've discussed it, we've given it more air and everyone is going to start sticking neomycin up their noses before they fly, Vincent.

**VR:** Yes, I'll notice them. They'll be, have the ones with the greasy nose.

**DG:** [laughs] All right. Let's jump right into COVID. Things are going in the right direction because I always see these posts like, "Oh, we're about to have another horrible surge." I'm not seeing that. We're down to, 5,000, 6,000 folks in hospital in the entire country. Unfortunately, we just had a gentleman come in last night, in the ICU, down to about 1,000, deaths. New deaths this last week, we actually, finally got under 100 deaths per day. Kind of crazy that we're all excited about that, this rate, but, 36,000 a year at that rate, if you do the math, but, we are going in the right direction.

You look across the country, I'm not seeing any hotspots. Everyone is 2% or less, 1% or less of deaths across the country. Then if you look at the wastewater, we're really about as low as we've ever been over the last year. We're at June levels from last year, really come down pretty nicely here. We'll keep track and see. One of the tough things is that the gentleman that I was just talking to today who got admitted, he went to a big concert. This is actually his third time getting COVID. Went to the big concert, it was Sunday night, enjoyed it.

It was sort of an Irish classic rock band, so we chatted about that. Then it was like three days later, started to feel sick, started to feel crummy. Goes to one of our local hospitals, maybe not the best of our local hospitals. Basically, because he feels crummy and falls down, they do a CT of his head, or they want to do CT of his head. He's like, "Listen, I'm here because I feel crummy, and I'm having trouble breathing. I have a cough. If you want to do a CT, you should

do it in my lungs." Anyway, he leaves there. He leaves there without the proper diagnosis, ends up at one of our urgent cares, sends him to the hospital where I spend a bit of my day.

After Columbia this morning, I was at this other hospital where finally someone actually did a test. He's got COVID. He relates that the last time he got COVID, he got started on some medicine. Apparently Paxlovid, he tells me. He started that within a day or two of symptoms, said it was a breeze. He felt better, he felt great. Now he's actually in the hospital on oxygen and not doing so well this time. It's still out there. Maybe part of the numbers is that people are missing diagnoses. This guy could have easily been missed. Let's talk about testing.

This is the *MMWR*, "SARS-CoV-2 Viral Shedding and Rapid Antigen Test Performance - Respiratory Virus Transmission Network, November 2022 - May 2023." This study looked at 354 participants in 129 households. Participants who were enrolled in this household transmission study completed daily symptom diaries and collected two nasal swabs. They're going to do, SARS-CoV-2 RT-PCR. They're going to do culture antigen tests. They're going to do this each day for 10 days after enrollment. I'm going to give you some numbers, then we're going to look a little closely at those numbers.

Antigen test sensitivity was calculated using the RT-PCR and viral culture as the references. The peak percentage of positive antigen, 59%, RT-PCR, 83%. Those are going to occur three days after onset and the peak percentage of positive culture results, only 52% occurred two days after onset. Now, they're going to tell us, and this is of course, what's going to end up in the media, they're going to say the sensitivity of antigen tests was 47%, and 80% using RT-PCR and culture respectively as the references. A couple things that, I think, if we look closely at the figures, a big thing, and this is something we've talked about over time.

This is not new. It's that there's a big issue if you're asking about symptomatic versus asymptomatic. When you look at the folks, and there's this nice figure, we've got panels like A, B, and C. Panel B, which is great is, OK, so it's about two to three days, you are symptomatic. We're talking about a 70% positivity rate. If we're talking about symptomatic and actually ends up with a fever, then actually, our sensitivity is up there at about 80% plus. Not so bad in the context of, I've got a fever, I've got symptoms, versus I'm just trying to pick up an asymptomatic person who may not have a lot going on.

**VR:** It's quite a striking difference between the symptomatic and asymptomatic, right?

**DG:** Yes, it really is. I think that's important. You see this 47%, oh my gosh, antigen tests don't work anymore. As we've always talked about, they work in someone who's febrile and symptomatic. They're not a great test for, oh, you've been exposed and let's try to catch a positive low level.

**VR:** Basically, probably that is because the viral load is lower in an asymptomatic person, do you think?

**DG:** I would have loved if they had another figure where you just said, what's the sensitivity, as we've talked about over time, relative to this viral, so, the copy numbers, et cetera.

**VR:** Unfortunately in panel A, they have culture positivity only, which is not really useful. I would like them to do a plaque assay. Why can't we do that? Give me a titer. I want to see how much virus is present at different days after onset, right? That would be very useful.

**DG:** Yes. Even, yes, as we mentioned, quantification of all these things, not just this binary.

**VR:** Right.

**DG:** COVID active vaccination, we've talked about for a while, that CDC has recommended for folks 65 and older, get a shot. That was recommended February 28th. For this section, we have the short communication published in the journal, *Vaccine*, "COVID-19 Booster Vaccine Uptake and Reduced Risks for Long-COVID: A Cross-sectional Study of a U.S. Adult Population." This study examined associations between booster uptake and Long COVID prevalence among 8,757 U.S. adults aged 18 years or older with a history of COVID-19 infection from the 2022 national health interview survey. We're dated there a little bit.

Weighted prevalence and logistic regression models examine relationships between self-reported COVID-19 booster, vaccine status, and Long COVID, adjusting for socio-demographics and health factors. Individuals receiving the COVID-19 booster vaccine had a 25% lower adjusted odds of Long COVID compared to the unvaccinated folks or I should say the unboosted, is really the proper.

**VR:** This is not the booster. It's the new vaccine, the Omicron-related vaccine. Correct?

**DG:** If you look at the dates, yes, it was basically getting that vaccination, yes. The language is confusing when you think about boosters because now we don't call it a booster, we call it the new vaccine, but it's the new-new vaccine. I don't know how much this is going to translate. There may be a diminishing returns, and I know we talked about a study last week about, with kids. Oh, the kids hadn't got a vaccine or had been certain period out from the vaccine. Was that a vaccine effect? Was that a new variant effect? I thought it was important to throw this in there because a lot of people at this point, why are they even getting a booster?

It's because they're not thinking they're going to die. Many of us are still as immortal as I was when I tried to climb Half Dome. But we don't want Long COVID. I don't want to be alive and chronically fatigued, and cognitively impaired.

Passive vaccination, still waiting for a little more information on Pemgarda. That's the new Evusheld. We'll give people updates when we actually start seeing this being used and accessed, the rest.

COVID early viral phase. Unfortunately, the gentleman that I saw today missed that first-seven-day window. We have NIH treatment guidelines. We have IDSA guidelines. Feel free to share those. Then, if your provider is not familiar, just ask that you get NIH, IDSA, ID Society of America guideline therapy.

A couple things I want to comment about. Maybe this relates to our opening. WHO reports major overuse of antibiotics for treatment of COVID-19. There was that presentation at ESCMID Global. This used to be ECMID for those people that - This is the annual meeting of

the European Society of Clinical Microbiology and Infectious Diseases. We get a quote from one of the presentations. "When a patient requires antibiotics, the benefits outweigh the risks associated with side effects or antibiotic resistance," says Silvia Bertagnolio, MD, WHO unit head for surveillance, Geneva, Switzerland.

"However, when they are unnecessary, they offer no benefit while posing risks, and the use contributes to the emergence and spread of antimicrobial resistance." I think this is important. There's always this, people are ready to criticize, "How come you are withholding those life-saving antibiotics?" are much less likely to realize that antibiotics used inappropriately can be associated with harm. At the same conference, we have the "Antibiotics Have No Beneficial Effect on Clinical Outcomes in Patients Hospitalized with Moderate COVID-19."

Actually, let's read what they found, and then maybe we want to redo that title. At this meeting, they presented an analysis of over 1,300 adults from Germany who are hospitalized with moderate COVID-19, where they found that treatment with antibiotics was associated with a five times greater likelihood of COVID-19 deterioration compared to patients who did not receive antibiotics. Maybe that should be, "Antibiotics are Harmful," because that's not just innocuous. That's not just failing to have a beneficial effect. That's five times greater likelihood of deterioration. That's not good.

**VR:** Still, many physicians give Z-packs, right, all the time for COVID?

**DG:** All the time. Unfortunately, I think the data we've talked about, the majority of people with COVID are still getting antibiotics. People are still throwing antibiotics at them. it's crazy. We may need some sort of revision about who's allowed to use antibiotics because they're being misused. You wouldn't let people just give out chemotherapy without having gone through the training to understand the risks and benefits. Maybe your primary care doctor shouldn't be just giving out antibiotics when we keep sharing evidence of greater harm when you do this.

Actually, you're harming your patients. Think about that. They may demand them, but you wouldn't do something harmful to your patients knowingly. Hopefully, we're educating some people. What do you do? Number one, Paxlovid. Number two, remdesivir, molnupiravir, convalescent plasma in certain circumstances, and then, of course, isolation guidance so that we don't get everyone else sick. Then week two, this is interesting. I was seeing this patient today with a colleague of mine, Alek Shalshin. We've weathered the storm of COVID together for the last few years.

I see this patient. When I go in the room, the patient is not on oxygen, but the chart has him on 2 liters of oxygen, but his saturations are in the 90s. I see that he's been started on steroids. I asked the nurse, "Is he on oxygen? Is he not on oxygen?" Apparently, he's not on oxygen, but he's being charted as on oxygen. We go ahead, make sure he's not on oxygen. I say, "Listen, I'm not going to give him steroids unless he really is hypoxic." We get an oxygen saturation of 90% on room air. Yes, that oxygen should actually be turned on. As we say, during that second early inflammatory week, steroids at the right time, in the right patient, at the right dose.

This is after the first week, and in patients with room air, oxygen saturation is less than 94%, like our gentleman here. Anticoagulation guidelines, pulmonary support, and remdesivir still has a role, if in the first 10 days from symptom onset, not on a ventilator. Immune modulation. We haven't talked about this in a while. We talked a bit about tocilizumab, but now we have the article, "COVID-19 Immunologic Antiviral Therapy with Omalizumab (CIAO) - A Randomized Controlled Clinical Trial." What is this? Omalizumab is an anti-immunoglobulin E monoclonal antibody.

You're trying to neutralize, get rid of that IgE. This is used to treat moderate to severe chronic idiopathic urticaria, that's hives, asthma, nasal polyps. Here they're investigating the idea that this omalizumab may enhance the innate antiviral response and have anti-inflammatory properties. These are the results of a phase II randomized double-blind placebo-controlled trial where they compare treatment with placebo in hospitalized patients with COVID-19. The primary endpoint was the composite of mechanical ventilation and/or death at Day 14, but there are also secondary endpoints included, all-cause mortality at Day 28, time to clinical improvement, duration of hospitalization.

Ultimately, it's a small study with only 40 patients. You get 20 getting drug, 20 on placebo. On Day 14, three, 15% of patients from the treatment group, but double that six or 30% from the placebo group have died or received mechanical ventilation. Numerically fewer adverse events were reported, actually, in the treatment group. No drug-related serious adverse events. They have one of these nice survival probability Kaplan-Meier curves where it's really interesting. You can see when the deaths occur, but it's not as impressive as the numbers because you'd see like, basically you get out to the first few days, and then there are a number of people that die in the placebo group.

Then you get out to like Day 12, same number, so the people just died a little bit later. Then you go out to Day 22, 24, comes together. Then it's about Day 25, 26 when you actually see a few more people in the placebo group die, which is really interesting because when you get out to about Day 28, a lot of people out there is, you've survived, they're getting ready to clap you out or something. Actually, you're seeing this late mortality.

**VR:** Yes, it's not impressive at all.

**DG:** Not impressive. Small study, but probably something that'd be interesting to know more about, be interesting to see if they move forward with more trials.

**VR:** Yes, a bigger study would be good, right?

**DG:** We also have the article, "Abatacept Pharmacokinetics and Exposure Response in Patients Hospitalized With COVID-19 - A Secondary Analysis of the ACTIV-1 IM Randomized Clinical Trial," published in *JAMA Network Open*. What is this drug? Trade name, Orencia, it's a recombinant fusion protein that inhibits T-cell activation, thereby reducing multiple inflammatory cytokines, including interleukin-6 and tumor necrosis factor alpha, that are part of the COVID-19 cytokine storm. I probably should mention, we call it the cytokine storm, and I like that name.

Is interleukin-6 higher than we see in bacterial sepsis? No, but I think, it still helps to keep people thinking. This is not the second rebound week. This is the second inflammatory

cytokine storm week. Here, these investigators conducted a planned secondary analysis of this trial with the goals to, one, look at the pharmacokinetics of this agent, relate exposure with clinical outcomes, determine the need for dosage adjustments, like, are they even really getting to the target they want? In the secondary analysis of the pharmacokinetics and exposure-response data for 395 hospitalized patients who achieved this higher projected abatacept exposure, they noticed significantly reduced mortality, a higher probability of recover, and fewer composite safety events.

The clearance and exposure is related to total body weight, baseline disease severity. Again, if you actually look at the data, a statistician is going to tell us it's different, but there's really big overlaps here.

**VR:** Yes, the two means are about the same.

**DG:** Statistically, a statistician is going to - Yes, and that's always what is a problem as a clinician, Is it clinically significant? Is this statistically significant? Is there really a difference if we'd done a trial with a really high dose? Maybe this will be something that fuels further investigations. We're going to spend most of our time, I think, today on late phase, PASC/Long COVID. I was actually just watching a new Netflix last night, some new Netflix gut microbiome show, by the way, so, for our listeners if they're interested. The article, "The Gut Microbiome Associates with Phenotypic Manifestations of Post-acute COVID-19 Syndrome," recently published in *Cell Host & Microbe*.

I must admit I'm still trying to wrap my head around the evidence and the reports from my patients about the profound impacts of modification of the gut microbiome on symptoms and even biochemical abnormalities associated with PASC. Here, a total of 1,207 Hong Kong Chinese with post-acute COVID sequelae, so they call it PACS instead of PASC, were recruited in two cross-sectional cohorts. We've got, 1,011, and we've got this longitudinal cohort of 196. Clinical phenotypic data, 94 factors were collected including demographics, comorbidities, medications, diet, COVID-19 history, vaccination records, symptoms.

Then they perform this metagenomic sequencing on the collected fecal samples. Reminds me, Vincent, of our conversation at that New York Yacht Club dinner, about this sequencing, and exploring the microbiome in these folks. Now, they use this information to develop a machine-learning model for using the microbiome to predict specific symptoms. They looked at 585 bacterial species and 500 microbial pathways, which they report explained 12.7% of the inter-individual variability in the symptom, post-acute COVID symptoms. Three gut microbiome-based enterotypes were identified in subjects with post-acute COVID symptoms and associated different phenotypic manifestations.

The trained model showed an accuracy of 0.89 in predicting individual symptoms of post-acute COVID symptoms in the test set and actually had a sensitivity of 86%, specificity of 82% in predicting upcoming symptoms in this independent longitudinal cohort. Now, this is one of those, I see this and I'm so excited but I can't get access because it doesn't get updated on the Columbia access until two days later, and now I'm all excited to dig even deeper into this because I want to know, what are the microbes? What are we seeing here? I had in my head, am I going to see that the *Bifidobacterium* is depleted because that's what we're trying to put back in, in our therapeutics?

Yes, the top-ranked gut microbiome features included depletion *of Bifidobacterium adolescentis* and R*oseburia hominis* and enrichment of C*lostridium bolteae* and *Flavonifractor plautii* and the urea cycle. There's not only gut microbes, we're actually seeing some other biochemical features as well.

**VR:** Not clear if it's an association or causation, right?

**DG:** That's clearly true. Yes, clearly true. Ideally, what we want to do, and we're going to have the opportunity to do this, people who are listening, of looking at people's microbiome, and then they get COVID and then you look afterwards and you see, was this change? Even better, then you restore that pre-infectious microbiome. Yes, because I hate just shooting in the dark, hey, take 10 billion twice a day of the *Bifidobacterium*. I left it then, are we achieving anything? Are we restoring the microbiome? Is that the right dose for you?

Then correlating our interventions with restoration of microbiome, and actually, resolution of symptoms. We have the article, "Characteristics and Determinants of Pulmonary Long COVID." It was published in *JCI Insight*. I'm always searching for objective abnormalities that both validate, explain the symptoms and abnormalities seen in people with PASC. Here, the author shared the results of a single center retrospective study that included 1,097 patients with clinically defined Long COVID, characterized for persistent pulmonary symptoms, so, trouble breathing, cough, chest discomfort, had to last at least one month or longer after resolution of the primary COVID infection.

They ultimately end up with 929 patients with post-COVID pulmonary symptoms. They measure pulmonary function tests, stratified diffusion impairment, and restriction as measured by predicted diffusion capacity for carbon monoxide and total lung capacity. Dyspnea was the predominant symptom in the cohort; 78% had similar prevalence regardless of degree of diffusion impairment or restriction. Don't worry, I'm going to explain what does all that terminology mean. What did I just say? What are we talking about here? Let's just go through.

The DLCO, so, diffusion capacity for carbon monoxide, this is a way of really measuring, is there good gas exchange in the lung? You're looking at the diffusion here. Then pulmonary restriction is, you have a total lung capacity, a predicted total lung capacity. You take a big deep breath, how much can you fill those lungs? If your total lung capacity is less than 80% of what you'd expect, you would call this a restricted. We can have restrictive patterns, we can have normal patterns. Obstructive is you can fill those lungs, but it really takes you a long time to get it back out because the flow is obstructed.

Then a sub analysis of CAT scan imaging identified radiographic evidence of fibrosis in this patient population. What are we seeing here? In some cases, you're actually seeing fibrosis. You're seeing objective CT imaging evidence of basically scarring fibrosis. Also, in some patients, I will say, not all patients, but some patients, you're actually seeing there's impaired gas exchange. You're also seeing that the lungs are not able to expand to that pre-infection capacity.

I think we're coming down to the home stretch with the last article, “Long COVID: Plasma Levels of Neurofilament Light Chain in Mild COVID-19 Patients with Neurocognitive Symptoms,” published in *Molecular Psychiatry*.

It's a good one to wrap things up this week. It made me think of one of the last *This Week in Neuroscience*, where you guys were talking about these organoids, right, and exposing them to virus?

**VR:** Yes.

**DG:** I'm on the same page with you, Vincent, I'm not sure it's exposure to the virus, but exposure to some sort of inflammatory milieu that gets triggered. The most frequent symptoms of this spectrum of cognitive issues, chronic fatigue, neuropsychiatric complaints, nuance of depression, anxiety, headaches, dizziness, disorders of smell and taste. Several mechanisms have been proposed to explain the neuropathogenesis of Long COVID, including active viral replication in the CNS, immune activation secondary to systemic inflammatory responses. Maybe the data is most supportive of that.

Spike protein damage to the endothelium and perivascular inflammation, microvascular injury, hypoxic consequences of severe disease, certainly see that in some cases. What is this neurofilament light chain? Plasma neurofilament light chain is a highly specific structural proteins of neurons. It's been validated as a biomarker for neuroaxonal damage. Not going to tell us how, but it is going to tell us if there is neuroaxonal damage. Neurons, axons, are these long projections that are part of the transmission of signaling.

Are those being damaged? Are we seeing evidence with this plasma neurofilament light chain? In this study, they're going to measure this as a biomarker of brain injury in non-hospitalized Long COVID patients. They get a group of 63 Long COVID patients, ranging from 18 to 59 years old, submitted to this neurocognitive battery assessment, then subdivided into different groups according to results. Plasma samples are collected during the Long COVID assessment and used for the measurements. Long COVID patients with cognitive impairment and fatigue symptoms presented higher levels of this marker when compared to Long COVID patients without these symptoms.

Again, they get some nice p values, and correlation analysis showed that levels of cognition loss and exacerbation of fatigue had a significant correlation with the higher levels. There is some degree of overlap, but there really are a number of Long COVID patients with really high levels compared. That's lots of overlap here. Interesting, but adding to our story.

**VR:** All right, so -

**DG:** I will close this. Yes, Vincent, you want to jump in there?

**VR:** These are all very small differences between groups, and just highlighting, I think, there's a great heterogeneity in this condition. It's going to be very hard to pin down.

**DG:** I think that's really true. There's no cut-off here where you can say, "OK, you have some cognitive issues. You had COVID. Oh, the test is above here. We only see that in folks with post-COVID neurological issues.” Yes, it's not going to be that biomarker, that definitive test

we're looking at. It's not like these serotonins of 12 that we're seeing. All right. No one is safe until everyone is safe. We're in a new fundraiser, Vincent.

**VR:** Right.

**DG:** We just entered the Floating Doctors Fundraiser, where for May, June and July, we're going to double your donations up to a potential maximum donation of $20,000, and a portion of these funds are going to go to travel - No, not to travel awards, they're all going to go to helping Floating Doctors [laughter] with their tremendous work that they do down in Panama.

**VR:** It's time for your questions for Daniel. You can send yours to daniel@microbe.tv. David writes, "You two suggested -" When people talk like that, it's not nice, right? "You two."

**DG:** It's hard to know, right? How do you pull tone out of this? I don't know. It says, "You have suggested, you two."

**VR:** "You two."

**DG:** "You two..,"

**VR:** I don't know. Usually, people are yelling at me when they say, "You suggested." "You two suggested that the apparent persistence of SARS-CoV-2 as detected by PCR could either be viral remnants or ongoing replication. Could these be distinguished by sequencing the PCR products over time? Continuing replication should result in a progression of accumulated mutations while viral remnant sequences would be unchanged."

**DG:** Yes. David, this is a reasonable question. I think what we've basically said is we don't know. If you find antigen, if you can replicate some RNA, OK, so you know that, and that's what you know, but is there a replication-competent virus? How are you going to do that? One is, actually, I think this is interesting if you could do periodic sequencing over time, and you actually see that there are changes in that sequence over time. That would be interesting. That would support more that there is ongoing replication. If the sequence is changing, why is it changing over time?

It would suggest it but doesn't necessarily tell us if that's true. It may just be that you're picking up different ones over time. Really, the key here, and it's been years of people trying to do this, is you do a biopsy, and some of these are gut samples. You do a biopsy, and then you grow up the virus? We're getting better and better at growing up the virus. If there's replication-competent virus there, which would be important to know, this is, we don't know, not, we know not, then that would be great. Vincent, thoughts on -

**VR:** Yes. I think this is an interesting approach, but it would just be better to culture the virus to quantify it because if it's replicating, you should see it, and you should be able to see how many PFU are there. It's very few people are doing that.

Sharon writes, "Dr. Griffin, the latest COVID vaccine guidance from the CDC seems to focus on older folks and the immune-compromised continuing to be vaccinated with the most updated shots. Are there some circumstances where younger people should get the latest

COVID vaccine? My nephew and his wife, 36 and 40, are traveling to Peru for vacation this June.

Both have had COVID twice, have had three previous vaccines. My nephew's wife takes a biologic for rheumatoid arthritis, but other than that, no major health issues. Would you recommend they receive an updated COVID vaccine before traveling, or should they ride the wave of hybrid immunity?"

**DG:** [laughs] I like the vision of riding the wave. It is interesting. We will often recommend certain vaccines for travelers out of season. Influenza, sometimes someone is like, "Hey, it's June and I'm traveling to the Southern hemisphere." We're like, "Right there, you might be moving yourself into the flu," and so we might give them a flu shot then. Still, when they get back, they'll get their flu shot in November. There is a little subtlety here to think about. Now, I say this in the context that it's a licensed vaccine, so individual may make a risk-benefit decision.

Part of it also is your interest and willingness to take vaccines. If you look and you say, "Hey, it looks like they're really starting to have, not a wave of hybrid immunity, but a wave of COVID infections in the area I'm heading to, someone is immunocompromised," you may start thinking about that, but yes, as we've talked about, the last CDC guidance was a little bit soft. Not a lot of people have gone with that. Just all the complexities there.

**VR:** Lisa writes, "I'm hoping you will take my questions. I've had it for a long time. Forty-seven-year-old, healthy healthcare worker, no risk factors for severe COVID. I had original two-vaccine series with Moderna in 2021, one booster later that year. Original strain. I work in an outpatient setting and see mostly well patients. I'm confident I've not had COVID. I'm still masking in public almost all the time. Have only been ill once with a cold in 2020, all household COVID negative at the time with repeating testing through Day 5. My question is, is three doses of the original strain mRNA vaccine still effective against severe COVID, specifically for people who have not had COVID infection.

Do we have data on this? What about effectiveness against COVID-related effects like myocarditis, VTE, et cetera? Because all vaccines and medications have potential side effects, although rare, I prefer not to vaccinate for COVID again without proof that it is needed for me. I'm trying to weigh benefits versus risks, and I don't know what the benefit is. I do know that my personal risk from COVID was low, at least after my three doses. I don't personally feel the benefit of avoiding infection for a few months is sufficient alone to get me more boosters.

I will, however, get another dose if there are data that the protection of the original three doses against severe disease has waned significantly. I've been unable to find these data. It seems it's assumed everyone has been exposed to post-Omicron strains, with the vast majority of the population having probably been infected by now, it seems that whole population data on vaccine effectiveness may not apply to 'NOVIDs' necessarily, for me to weigh my own personal risk here. I feel I really need data on those who have neither had COVID nor updated vaccine doses. Does it exist?" Didn't we talk about such a study last time, Daniel, the waning with the original vaccine?

**DG:** Yes. It is a challenge. I think the original idea was you get your three shots, that's going to give us your ongoing 90%. Then, when you compare getting a boost or not, you're really comparing it to protection on top of prior infection, on top of prior vaccination. What can I say to you? We're, right now, as I mentioned, lowest levels of COVID in wastewater and diagnoses and hospitalizations and deaths that we've seen since last summer. Right now you don't have to worry about this in certain ways, or I should say right now your risk is very low.

Really, I think the question is going to be, what are you going to do next October-November when rates are going to go back up? Are you going to keep masking? Are you going to think about doing a vaccine at that level? I'm getting from this, the fact that you've done three doses, you're not exactly particularly bullish on vaccines, so you prefer to avoid if possible. A lot of the data you're asking for, I think, we've talked about some of it, but yes, there are still some 'NOVIDs' out there like you. Then you've got to weigh, what are my risks of getting COVID? You'll be able to assess that next fall versus really the low risk of an adverse issue with the vaccine.

**VR:** Ellen writes, "Dear Daniel, you and others have cautioned against consuming raw milk due to the presence of H5N1 in dairy cows. However, not many Americans have access to raw milk, while many are consuming raw cheeses. Should the same caution apply?

**DG:** Ellen, this is actually a good question. Actually, it reminded me of the last *This Week in Microbiology*, Vincent, that you missed.

**VR:** That's right.

**DG:** Michael Schmidt and the crew did it without you, but they had your nice intro in the beginning, so it was almost like you were there. [laughter] What happens here? Raw milk, it's straightforward. I will admit, I have consumed raw milk in my lifetime, up in the mountains of West Virginia at some little farm. One percent of Americans, do you believe that, one percent of Americans, millions of Americans consume raw milk on a regular basis. Right now, maybe not the best time to be doing that. What about raw cheese? What about making cheese from that raw milk, never putting it through any pasteurizing option?

I would have the same concern. My initial thought was, "Oh, you see cheese, it goes through this whole process. That's why we make cheese\ because it's a way of preserving." Until we find out otherwise, maybe not so great for the moment.

**VR:** How much of that milk from those cows has entered the cheese chain yet?

**DG:** Yes, because it takes time to make cheese, other than a cottage or those quick cheeses. Most cheeses take quite a while.

**VR:** I think that the farms should be aware of whether they have H5 or not and take that into consideration.

**DG:** Yes. It may be more prevalent, right, as we've talked about?

**VR:** Yes.

**DG:** Maybe underdiagnosed.

**VR:** All right. Nikki writes, "Our point of care Cepheid PCR machines are set up to detect influenza A, B, RSV, and COVID. They do not reveal the subtype. If a patient walks in with H5N1, would we have any way of knowing? Also, we don't swab patients who present solely with conjunctivitis. How was avian influenza diagnosed in the Texas rancher?

**DG:** Yes, you bring up a good thing we probably have missed. I think there was a recent study, I don't know what to make of it yet, I'm trying to mull this over. It was where they had a 1% or 2% positive serology in a sampling, saying, "Oh, we're missing H5N1, and look, 1% to 2% of this population had it." If H5N1 is something that we see is affecting a herd and then we've got, one of the workers comes in with conjunctivitis, OK, you think about it, and then you go down this road. No, I think that we probably have missed some H5N1. The fortunate thing, if you're not a cat, and I actually really like cats, even though I'm maybe a dog person, I'm also a cat person, the cats are dying. We are fortunate so far in that we're not actually seeing severe disease in humans. We're just seeing this conjunctivitis. Vincent mentioned on one of our prior episodes that it had actually to do with the different receptor binding of H5N1 in avian versus the H1N1, the typical human influences.

**VR:** How was it diagnosed in the Texas rancher? That's a good question. I assume they do oropharyngeal swabs, right?

**DG:** You can do oropharyngeal. You can actually take a swab because when they've got this conjunctivitis, you get just all this fluid. The fluid, with a soft swab, you can actually collect that fluid.

**VR:** Or nasopharyngeal. Right.

**DG:** We'll do that sometimes. I've got a patient in the hospital now with a herpes infection. It just ends up with all this stuff. Also, sometimes bacterial, we'll end up getting a swab as well.

**VR:** What do you do for influenza? Nasal or oropharyngeal?

**DG:** Normally, for our influenza, particularly the Cepheid, we're going to do a nasal. Sometimes it can be a deep nasopharyngeal, or it can just be doing an anteriorness is probably just fine. Then here, with the Texas rancher, if you just get a little bit of that conjunctival fluid on that swab, you can send it off for a molecular test for the H5N1.

**VR:** That's *TWiV* weekly clinical update with Dr. Daniel Griffin. Thank you, Daniel.

**DG:** Oh, thank you. Everyone, be safe.

[music]

**[00:51:59] [END OF AUDIO]**