This Week in Virology

TWiV 1152 Clinical Update

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Guest: Daniel Griffin

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

(Music)

VR: From *MicrobeTV*, this is *TWiV, This Week in Virology*, Episode 1152, recorded on September 26, 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: With a mystery bow tie, some kind of molecule.

DG: It's Creutzfeldt-Jakob disease again with the prions on there.

VR: Yes, the misfolded proteins.

DG: Yes, yes. Well, I don't know how new it is, but there's a test now where they actually take the misfolded proteins and then they put them with the regular, the native proteins. It's almost like a PCR, it's like a chain reaction. All the other proteins misfold.

VR: Yes, it's called the shaking and quaking.

DG: The fast test.

VR: Yes, it's pretty cool.

DG: For those of you studying for your infectious disease board, that's the right answer. All right, let's jump into it. We have a lot today. I'm going to start with an entertaining quotation from Benjamin Franklin. “In wine there is wisdom, in beer there is freedom, in water there is bacteria.” I have my bacteria laden water here. All right, so we'll start off with mpox. Let me jump in with this. We have this article in a recent *CIDRAP*. I'm going to actually quote *CIDRAP* a couple of times. Going to shine a little light on this publication out of University of Minnesota.

CIDRAP published September 19 entitled, "Amid New Mpox Outbreak, Study Suggests Waning Protection of JYNNEOS Vaccine." I'm sure Vince will have some comments on the waning comment here. Here we read, JYNNEOS vaccine wanes significantly over the course of a year,

raising new questions about just how protected vaccinated people are against reinfection, and if booster doses of the vaccine are needed among at-risk populations. This study, which we're going to discuss, was released alongside an alarming development in the globalization of mpox.

More than 21,000 cases of virus have been recorded in the past year in the Democratic Republic of the Congo, UNICEF noted. On August 14, the WHO declared the DRC outbreak a public health emergency of international concern. In part, I want to comment, about 60% of cases are in children 15 years and under. The majority of the cases are in children 15 years and under.

Now, I know this led my cousin, who I will mention later as well, to ask, "Well, wait a second, how can all these children be getting it?" Now, in the DRC, children under age 15 account for about half of all the suspected cases and 80% of the deaths. How does that happen? How is this spread, Vincent. Isn't this like a particular sexually transmitted disease of a certain population?

VR: Well, it doesn't have to be. It can be close contact, which kids do with their parents.

DG: Yes, lots of close contact. We even think there's animals involved in the transmission. Yes, I just want to raise the concern here, because this particular clade of mpox that we're talking about, as we see, it's predominantly a disease of children. We're seeing more than 21,000 cases. We're seeing it spread to other countries. Yes, things are not going well with mpox in Africa. We read in Reuters that the number of mpox cases in Africa has surged.

Almost 200% compared to data a year ago. Deaths have increased 38.5% compared with the same period a year ago. As for the African CDC, “we can say today that mpox is not under control in Africa. We still have this increase of cases that is worrying for all of us,” Jean Kaseya, director general of African CDC, told a weekly briefing. I'll leave a link into that. What's this study?

Actually, it's a preprint posted on *medRxiv*. “Rapid Decline of Mpox Antibody Responses Following MVA-BN Vaccination.” That's the modified vaccinia Ankara, Bavarian Nordic vaccine. This comes out of Dan Barouch's group up in Boston. A little background here. Dan Barouch is someone I think that we rely on. I think it's just a matter of time before this is going to be a peer-reviewed publication. Here's where we are. The Orthopoxvirus genus consists of 12 viruses, including smallpox, mpox, vaccinia viruses.

Now, the replication-incompetent modified Vaccinia Ankara Bavarian Nordic vaccine, and this is JYNNEOS, was developed as part of the U.S. Strategic National Stockpile program for deployment in event of smallpox outbreak. The CDC has recommended this JYNNEOS vaccination to protect against mpox during the 2022 Clade 2b mpox outbreak. Now, due to limited supply, the recommended JYNNEOS administration was modified from that half a mL subcutaneous to this 0.1 mL by the intradermal route.

Here, these investigators performed an observational study in 45 adults who received the JYNNEOS vaccine or had confirmed diagnosis of mpox infection at Beth Israel Deaconess Medical Center up in Boston. They assessed serum antibody and T-cell responses for 12 months. All right, so what's the story here? We're looking at vaccinated. They're also going to

compare it to people that had been infected. We're going to get data on both. I'm going to, of course, focus on the mpox vaccination folks.

They're going to look at serum antibody or they looked at serum antibody and T-cell responses - that'll make you happy, Vincent - for 12 months following either a two-dose or a one-dose vaccination delivered by either the subcutaneous or the intradermal route. They assessed median antibody ELISA titers to these different mpox, it's really monkeypox, virus antigens, M1R, B6R, A35R, A29L, H3L antigens. I say monkeypox virus because we can rename the disease pretty quickly, which we did to mpox.

Those viral taxonomists, we're still waiting on them. It's still the mpox virus. They looked at these ELISA titers, these antibody titers to these antigens, but they also looked at T-cells. They're going to measure interferon-gamma CD4 positive and CD8 positive T-cell responses. They're going to do that with intracellular cytokine staining assays. Now, we're going to walk through the data a little bit here.

Serum antibody titers following vaccination have been shown to correlate with protection against monkeypox virus challenge in non-human primates. Whereas the CD4 positive and the CD8 positive T-cell responses did not correlate with protection. Sort of suggesting, based on that, the potential relevance of measuring these serum antibody titers following vaccination in humans. Median binding antibody ELISA titers to mpox antigens peaked at week three, following two doses of the JYNNEOS, but then declined at 12 months.

They've got data here for the two-dose approach. They've got data here for the one-dose approach. Really similar, we get a peak, and then we get this, what we've referred to as a contraction, an expected contraction of those antibody levels. With the two-dose, you're going to end up with about a log higher or so median IgG titer, and you're going to see this contraction over time. Now, here's the other thing I can say. That's just total IgG. Now, here's the other. I think this is important.

Mpox serum neutralizing antibody titers. These are the IgGs that are neutralizing, were detectable in only a few participants following two-dose or one-dose vaccination. They actually show us the data for that. We don't get the T cell data. They just tell us. I guess that's going to come when they get accepted. They tell us low peripheral interferon-gamma. That's our signature Th1, memory CD4 positive, and CD8 positive T cell responses were detected to vaccinia-infected target cells by intracellular cytokine staining at nine months following two-dose and one-dose vaccination.

They give us these median CD4 responses, they give us median CD8 responses, but were not detected by these mpox peptide-specific ELISPOT assays, and they don't show us that data. They bring it all together. Vince and I are going to discuss what all this means. They conclude, taken together, these data suggest that protective immunity may be waning in individuals who are vaccinated with the JYNNEOS vaccine in 2022, and that boosting may be required to maintain robust levels of protective immunity.

VR: OK, what's wrong with this?

DG: You tell us, Vincent. What's going on?

VR: Immunization or infection, antibody levels always decline. That's the way the immune system works. Here they're looking at baseline, 12 months later it goes down. That's what you expect if you did this with SARS-CoV-2, influenza, polio, you'd find the same thing. The fact that there are antibodies at 12 months means there are memory B cells present in these people. What that means is when they get infected with monkeypox virus, they're going to make antibodies.

The titer will go up, and that will protect them against mpox, the disease. There is no reason. This is ignoring fundamental immunology. I'm really surprised at Dan, because he knows better. Antibody levels always go down. We depend on a memory response to protect you. We don't depend on high antibody levels circulating all the time.

The same thing happened with COVID vaccines, Daniel. After that first round of vaccinations, the titers were going down, oh, they've failed, they're waning, they're no good. Oh my gosh, it's all over again here. Folks, don't worry about it. I think the key here is clinically, are these people protected? I don't care about the antibody titers, really. I want to know if they're clinically protected, and that's not done here.

DG: Yes, I think that's really - so one is the sky is not falling. This is basic immunology. You vaccinate, you get a peak in the antibodies, they contract over time. The question is, and it's sort of disease-specific, like for instance, with meningitis, we need to keep those antibodies high, so we have to give periodic shots. A lot of other diseases, there's a certain amount of time for the memory cells to trigger the elevation of those antibodies.

That's really what we're going to need to track, is OK, so it's a year out, and we have a fair number of folks here in the United States that are a year out from last summer's vaccination. Are we going to start to see cases or does this become a once-a-year vaccination in certain populations? That's probably great for Bavarian Nordic, if it's a once-a-year, it's a little bit more of a challenge logistically. Who knows? Maybe the malaria vaccines that we've been discussing on other of our podcasts will need to be a yearly thing as well.

VR: I hope that upon peer review, the language is toned down, because to say that this may indicate that the vaccine is not working, that's not right. You can't make that conclusion.

DG: Yes. You sort of have to say, yes, antibodies are contracting as expected, as we always say, and then sort of we'll need to follow to see whether or not there's a clinical - the language, yes, could be even a little softer, I guess. A little more here on - people may not realize this. Up until I'm about to say this, Bavarian Nordic's vaccine was really approved just for adults, but now we hear the Bavarian Nordic JYNNEOS vaccine was approved into adolescents, so 12 to 17 years of age. What about under 12?

Well, Bavarian Nordic is preparing for a clinical trial to assess the immunogenicity and safety in children 2 to 12. That's what we need. I think we talked above that this is primarily affecting children, so we want to look at the immunogenicity and safety in children 2 to 12 years of age. This will probably be a bridging study where you make sure it's safe, you make sure you get the bridging, meaning you get these antibody responses. This trial is partially funded by the Coalition for Epidemic Preparedness, so that's CEPI, Preparedness Innovations.

That's hopefully going to start in October. All right, it gets better or worse. According to media reports that cite government and health sources, India has reported an imported Clade 1b mpox case, making it now the third country outside of Africa to report this clade. The patient is a 38-year-old man from the Malappuram district in India's Kerala state who traveled from the United Arab Emirates, so Sweden, Thailand, and now India.

To close this section with maybe some more good news, I don't know, mixed here, on Tuesday, the 24th of September, we heard that Bavarian Nordic gets $63 million U.S. government order for the vaccine. This is going to be a million freeze-dried vaccines from this Danish biotech company. More sort of support here, *CIDRAP* out of University of Minnesota had a news brief, they called it. I get these emailed to me every day, where they report that at the United Nations General Assembly meeting, I watched a little bit of this.

U.S. President Joe Biden announced new support for Africa's mpox response, including the 1 million vaccine doses and at least $500 million in further support. In the UN address, Biden called on other countries and groups to step up their help for Africa, and here I quote, "We call on governments, charities, and businesses to match our pledge and make this a $1 billion commitment to the people of Africa. The U.S. Department of Health and Human Services, the Administration for Strategic Preparedness and Response is donating the 1 million doses to the international response.

It added that it is lending Bavarian Nordic, the maker of the JYNNEOS vaccine, 200,000 doses to ensure commercial supply to the U.S. market without diminishing the company's ability to fulfill these international orders and donations. African health officials have estimated the region needs at least 10 million vaccine doses for the current outbreak response. A number of groups have also announced donations, including the European Commission, Japan's government, and actually Bavarian Nordic itself. People are stepping up. Now I was like, "What can we do?"

I was talking with, as I mentioned, my cousin, Peter Daytz, with whom I sail. I did donate to UNICEF, which I think we mentioned is the largest purchaser of vaccines and a major supporter of this effort. I'm going to leave in a link here in case others want to do that. Now since my cousin works in investment management, I asked him if it would be helpful to buy Bavarian Nordic stock, Vincent, because they're that Danish company making the JYNNEOS vaccine.

This seemed like something where I wasn't just giving away money, but maybe I was responsible investing, so I also bought some Bavarian Nordic stock. My cousin seemed to think this might be helpful. He waffled there, but maybe we've got listeners who have better-educated ideas. I'm certainly not trained in stock investing, and I'm hoping this helps the company, but who knows?

VR: I think it helps the stock market, right?

DG: I don't know. Maybe if the company, the stock goes up, they'll be more bullish on expanding, we'll get more vaccine. I don't know what I'm talking about. All right, let's move into COVID. This is one of those times, Vincent, where you may have to talk about how we

name the clinical update because people Google certain things. We're going to talk about the fact here that SARS-CoV-2 was a zoonosis and spilled over to market.

We're going to talk about the article, "Genetic Tracing of Market Wildlife and Viruses at the Epicenter of the COVID-19 Pandemic," published in *Cell*. Sort of that lead-in because people are like, when I Google “Paxlovid rebound,” I find articles about Paxlovid rebound. When I Google “the lab leak hypothesis,” I find all this misinformation. Maybe people need to Google “SARS-CoV-2 zoonosis.” A couple of highlights, and this really is a great article I have to say, and hopefully, Vincent and I can walk everyone through it.

They start off with some nice highlights. Highlight number one, of course, common ancestor of SARS-CoV-2 linked to Huanan market matches the global common ancestor. Number two, wildlife mitochondrial DNA identified in samples from stalls positive for SARS-CoV-2. DNA from raccoon dogs, civets, and other wildlife species detected in market samples. Genotypes of potential hosts were reconstructed for retracing animal geographic origins. Then we get a nice summary. They really do a good job on this article. Start off with the summary.

Zoonotic spillovers of viruses have occurred through the animal trade worldwide. The start of the COVID-19 pandemic was traced epidemiologically to the Huanan Seafood Wholesale Market. Here they analyze environmental qPCR and sequencing data collected in the Huanan market in early 2020. They demonstrate that market-linked severe acute respiratory syndrome coronavirus 2, SARS-CoV-2, genetic diversity is consistent with market emergence and find increased SARS-CoV-2 positivity near and within a wildlife stall.

They identify wildlife DNA in all SARS-CoV-2 positive samples from this stall, including species such as the civets, bamboo rats, and raccoon dogs previously identified as possible intermediate hosts. They also detect animal viruses that infect raccoon dogs, civets, and bamboo rats. Then combining metagenomic and phylogenetic approaches, they recover genotypes of market animals and compare them with those from farms and other markets.

This analysis provides a genetic basis for a short list of potential intermediate hosts of SARS-CoV-2 to prioritize for serological and viral sampling. They have a really nice, clear graphical abstract where they've got little maps where you can see, the SARS virus, the market sort of viral hotspot. Showing that not only are they actually identifying, where are all these viral samples, but even these potential intermediate hosts. They've got these cute little cartoons of the raccoon dog, the civet, the bamboo rat, and then a little bit about the DNA and the SARS-CoV-2 phylogeny.

I have to say, this is an important topic. My plan is to actually spend some time today walking through this. I've got a few other articles that are sort of on hold for next week so we could spend some time here. Let's go through, what we know so far and what this adds. We'll start off. When cases of COVID-19 were identified, the question of where the virus came from and how it entered the human population started to be asked. Now, by the summer of 2022, the science was compelling that SARS-CoV-2 had entered the human population through multiple transmissions from wildlife at a market in Wuhan.

Articles published during the summer of 2022 in *Science* Volume 377, “The Huanan Seafood Wholesale Market in Wuhan Was the Early Epicenter of the COVID-19 Pandemic,” “Wildlife

Trade is Likely the Source of SARS-CoV-2," "The Molecular Epidemiology of Multiple Zoonotic Origins of SARS-CoV-2," detailed the evidence around the timing and way that SARS-CoV-2 entered the human population. Within the Huanan wet market, the data statistically located the earliest human cases to one section where vendors of live wild animals congregated, and where virus-positive environmental samples concentrated.

It appears that infected animals were brought to the market and this is where the jump to humans occurred. Based on sampling and analysis, the first zoonotic transmission likely involved lineage B virus around the 18th of November 2019. The separate introduction of lineage A likely occurred within weeks of this event. These findings indicated that it is unlikely that SARS-CoV-2 circulated widely in humans before November 2019 and define the narrow window between when SARS-CoV-2 first jumped into humans and when the first cases of COVID-19 were reported.

Spatial analysis within the market showed that SARS-CoV-2 positive environmental samples included cages, carts, freezers. These were associated with activities concentrated in the southwest corner of the market. This is the same section where the vendors were selling live mammals including raccoon dogs, hog badgers, and red foxes immediately before the COVID-19 pandemic. Multiple positive samples were taken from one stall known to have sold live mammals.

The water drain proximal to this stall as well as other sewerages and a nearby wildlife stall on the southwest side of the market tested positive for SARS-CoV-2. There was an article, "Surveillance of SARS-CoV-2 at the Huanan Seafood Market," I'll leave a link, published in *Europe PMC* in April 2023 and we have covered this extensively. *TWiV* has covered this issue on several podcasts including the *TWiV* special, "How the Pandemic Began in Nature," in five key points. I'm going to leave in a link. There's actually been SARS-CoV-2 origin discussions on a number of previous *TWiVs*.

I'm going to leave in links to *TWiV* 1019, Eddie Holmes on SARS-CoV-2 origins. *TWiV* 1017, From Nature, not a Lab. *TWiV* 995, Viral Origin Stories, that's *TWiV* 995. Also *TWiV* 940, Eddie Holmes again, Eddie Holmes in On Viral Origins. *TWiV* 876, Spillover Market with Michael Worobey. *TWiV* 762, SARS-CoV-2 origins with Robert Garry. *TWiV* 760, SARS-CoV-2 origins with Peter Daszak, Thea Kølsen Fischer, Marion Koopmans. TWiV 774, Kristian Andersen, Robert Garry, and the deleted SARS-CoV-2 sequences. All right. We've mentioned this before.

VR: Yes, we have. This is an important extension though. They had new data, new analysis. Remember that the sampling wasn't optimal. The sampling of the market was done after it was closed and not really extensively. This is what we have and this is an extension of those samples and provides additional support for the notion, the hypothesis that the pandemic began here in the market, spillover from animals to people. It's quite clear.

DG: Yes. There's a lot of complexity here. Now that we've laid the groundwork, let's get ready to dive in. Vincent, you can help me because this is a complex *Cell* paper. We're going to start off with what do we learn from this paper. Number one, and this is complex, but this is important. SARS-CoV-2, so the virus, its genetic diversity linked to the Huanan market is actually consistent with market emergence. Here's what we read.

If the Huanan market was the site of origin of the transmission chains that led to COVID-19 pandemic, then the common ancestor of the market-associated viral genotypes should be equivalent to the common ancestor of the pandemic, if you can get appropriate sampling. To test this hypothesis, they assessed intra-sample variation of the SARS-CoV-2 environmental genomes from the Huanan market, assigned market sequences to virus lineages. Performed a phylodynamic inference to compare the genetic diversity of SARS-CoV-2 within the market to its genetic diversity globally.

They found that the most recent common ancestor, that's the MRCA, most recent common ancestor of market-associated genotypes was actually equivalent to the MRCA of the larger pandemic. They show us all this diversity as this Figure 1A goes through all this. That's the first part. They've got this most recent common ancestor analysis. Then they find increased SARS-CoV-2 positivity in and near a wildlife stall in the Huanan market. I've talked about some of this data before.

The Huanan market was sampled on multiple dates at the start of 2020 with different sampling trips having different purposes. They found that the rate of qPCR positivity was unevenly distributed within the market with increased positivity in the - if you've been following, southwest sections. One stall, wildlife stall A, stood out, with a 30% PCR-positive rate. Three of its 10 samples collected on January 12th, a cart, a hair/feather removal device, and a sample collected from the ground were PCR positive for SARS-CoV-2.

VR: I think this is important that this is a big market. Mammals were not sold everywhere and they were sold in this part, the southwest corner. That's where we see SARS-CoV-2 positivity.

DG: This is nice, southwest, nice map. They show us the hotspot. We get to see all the data here. Next, mammalian wildlife species detected in five SARS-CoV-2 positive samples from a wildlife stall. Let's go through what this is. Environmental samples with viral RNA can also contain genetic evidence of the mammalian host that shed the virus. We get a sample, we get the SARS-CoV virus, and then we can ask, "Hey, in the same sample, where did this come from? Maybe we can pick up the animal."

They developed and benchmarked a highly specific metagenomic pipeline for quantifying the abundance of animal mitochondrial DNA, let's say, mtDNA, mitochondrial DNA, in environmental samples. The five SARS-CoV-2 positive samples from wildlife stall A contain mitochondrial DNA from raccoon dogs, hoary bamboo rats, and dogs, so Canis lupus familiaris, and European rats. Amur hedgehog and Malayan porcupine mitochondrial DNA was present in four of the samples.

Reeves's muntjac and Himalayan marmot mitochondrial DNA was found in three, and one sample contained masked palm civet mitochondrial DNA. Of these species, raccoon dogs, rabbits, and dogs are documented as susceptible to SARS-CoV-2, with raccoon dogs experimentally confirmed as capable of transmission and in vitro evidence of civet susceptibility.

VR: I think this is very important. Mitochondrial DNA can be used to identify what mammals were there, and they could, and then among them are mammals that can be infected with SARS-CoV-2.

DG: They're really pulling this all together. Then this is confirmatory, OKy, so we've got SARS-CoV-2, we've got the animals. Now, does this stuff all work? What if we look for other? There should be other viruses that those mammals have. Really, I like this sort of a confirmatory analysis where they report that wildlife stalls and SARS-CoV-2 positive samples contained other mammalian viruses associated with the animal trade. The presence of animal viruses with predictable host ranges can provide evidence of animals productively infected with viruses in the Huanan market.

By mapping sequencing reads to a custom database of human and animal viruses with stringent filtering, they identified several mammalian viruses present in the market. Not only do they find the DNA of the viruses of the animals. Not only do they find the DNA of the animals, but they find the typical viruses that infect those animals. They're identifying things like the raccoon dog amdovirus, the civet kobuvirus, canine coronavirus, and the bamboo rat coronavirus, among others.

VR: I think that's a nice addition that these animals have other viruses. Can we detect them? Yes, we can.

DG: Yes, yes. You find SARS-CoV-2, you find all these sort of lineage that fits into this really nice common ancestor analysis, really showing that you had quite a bit of diversity. I'm trying to, the first sort of part of this thing like, oh, what's the virus doing there so close to this level? It's not the virus. There's this incredible diversity, actually, genetic diversity in this market, really consistent with a bunch of animals were brought there that were infected.

The lab people, they didn't just have to make, supposedly, a virus, they had to make like, what, all these different viruses with this great tropism for all these animals. The first part sort of falls apart. The second is you've got this really nice, hotspot, you've got the right animals in that hotspot. You confirm that with the fact that you're also able to detect other viruses that affect those animals.

Why do we spend so much time on this? If you look through the list of times, it's important because as we discussed, there's a lot of damage from this misinformation campaign. Really, it's a misinformation, anti-science campaign. In *TWIV* 1140, we actually discussed that in the clinical update.

VR: The evidence in supporting a natural origin of SARS-CoV-2 continues to accumulate and there's no support for any other origin. Individuals who promulgate other origins are not really being scientifically rigorous. It damages science, as Daniel says, because you generate mistrust for scientists in China, for example, which isn't justified in this case at all.

DG: Here's the information. It's out there. We keep learning more. Back in 2020, it was a reasonable question to say, oh my gosh, there's a lab nearby. A bat got brought there. Somehow there was a connection. It's overwhelming at this time. This is what happened. There's a lot of information here. All right. Moving into what is going on with COVID right now. Now North Carolina's the hotspot. Four to 5.9% of the deaths, total deaths in North Carolina are due to COVID.

VR: Oh, so Daniel, that must mean that it came from a lab in North Carolina, right?

DG: Well, my little brother lives in North Carolina and he's nothing but trouble. I'm going to call Ben up because maybe he's responsible. He has these big parties you see, Vincent.

VR: I see.

DG: I'm only joking, Ben. My brother had nothing to do with this. All right. What about the wastewater? It looks like things, a little bit of a blip there out in the West, but most things look like they're moving down with wastewater across the country. I'm predicting, Vincent, a lull coming up early November for about seven days before it shoots right back up in December and January. Vincent's predicting we're all good till next summer, right?

VR: I think so. That's my prediction. I could be wrong, but I want to inject some optimism into this story.

DG: All right. The variants, it's all Kp.3.1.1, but this is all JN.1 lineage. That'll be important when we talk about how well things go with the booster doses. A lot of people are jumping in for those booster vaccines. We seem to be like team Novavax in my family with my parents both got it on Monday. My wife got it. Couple of my daughters have gotten it. I don't know if my son in England can get it. If anyone's over there in England, let me know if there's access.

Nice thing is nobody had, well, any noted reactogenicity, which was really nice. Everyone's getting their updated vaccines.

All right. We will talk about, unfortunately, folks do keep testing positive, early viral phase. We still have our guidelines. Number one is Paxlovid, recommended by the ID Society of America, the NIH COVID-19 Treatment Guidelines. I actually had a patient - well, I was consulting on a patient in the hospital today, and she was telling me her story. It was about nine days ago that she was diagnosed at an urgent care with acute COVID.

They suggested she go get Paxlovid. She went. Apparently, the pharmacy told her that it would be $1,100 for her to get that Paxlovid. She wasn't really sure what to do. She ended up not getting the Paxlovid. She called her pulmonologist, who, as she explained, does not believe in treating Paxlovid with those medicines. Instead, he increased her steroids. Now I'm taking care of this hypoxic woman in the hospital. I informed her that I take care of a lot of that particular provider's hypoxic patients during week two in the hospital, and she got the point.

OK, number two, remdesivir; three, molnupiravir; four, convalescent plasma, and isolation guidance. I looked ahead. I saw we have some questions. We're going to talk a little bit about isolation guidance in our Q&A section. Number two, early inflammatory weeks. This is week two. This is for a lot of folks, that bad week when they might feel even more rotten than the first. When they get that hypoxemia, that cytokine storm, the early inflammatory phase. Steroids at the right time in the right patient at the right dose.

Number two, anticoagulation. Number three, pulmonary. Four, remdesivir. This is an updated data. I know sometimes we memorize, A, if A, then B. We learn more, and then A might become C. The article, "Lower Mortality Risk Associated with Remdesivir + Dexamethasone versus Dexamethasone Alone for the Treatment of Patients Hospitalized for COVID-19," published in *CID*. The reason this is relevant is for a while, we sort of had this dogma of, "OK,

if it's past day 10, if they're in the ICU, if they're requiring a high amount of oxygen or ventilated. OK, maybe you missed your window for remdesivir."

Here is this issue about, "Hey, maybe if it's that second week and they're on dexamethasone, let's see. Adding the remdesivir, is that helpful or not?" Large multicenter U.S. hospital database was used to identify hospitalized adult patients, with a primary discharge dose of COVID-19. Also, they flagged it as "present on admission," treated with remdesivir and dexamethasone or dexamethasone alone from December 2021 to April 2023. Patients were matched one-to-one using propensity score matching, stratified by baseline oxygen requirements.

They do this Cox proportional hazards model to assess time to 14- and 28-day in-hospital all-cause mortality. A total of 33,037 patients were matched. Most of the patients were 65 years of age or older, so 72%; 78% were white and non-Hispanic. Remdesivir plus dexamethasone was associated with lower mortality risk versus dexamethasone alone across all baseline oxygen requirements at 14 days.

Looking at no oxygen, we saw an adjusted hazard ratio of 0.79, so 21% reduction. Low flow, 0.70, so 30% reduction. High flow oxygen or non-invasive ventilation, 0.69, so 31% reduction. Invasive mechanical ventilation, ECMO, also 0.78, so 22% reduction. All this is with confidence intervals, favoring this. Similar results at 28 days.

VR: It's consistent with other studies showing that you can give remdesivir in-hospital. It's not too late. Right?

DG: Yes. We certainly do it in the hospital. The little twist here was we often said, "If it's after day 10, if they're already in the ICU -" and this was like sort of just straight remdesivir studies, you seem like you'd sort of missed your window. It looks like if you've got them in the ICU, you've got them on steroids, it's still worth doing the remdesivir.

All right. Just a little bit here, a little disturbing bit here on the late phase, PASC, Long COVID, because we've got a really nice - It's actually another *Cell* paper on Long COVID that we'll be talking about next week, among some other things. I don't know if you saw this one, Vincent, but this one really bothered me. This is the article, "Changes in Memory and Cognition During the SARS-CoV-2 Human Challenge Study," recently published in *eClinicalMedicine*. I don't know if our listeners remember that human challenge trial, we had some trepidation about giving the SARS-CoV-2 virus to healthy volunteers.

We didn't really fully understand the repercussions. Here, 34 young, healthy, seronegative volunteers were inoculated with wild-type SARS-CoV-2 under this prospectively controlled conditions. Volunteers completed daily physiological measurements and computerized cognitive tests during quarantine and follow-up at 30, 90, 180, 270, and 360 days out for a year. Linear modeling examined differences between infected and inoculated but uninfected individuals.

The main cognitive endpoint was the baseline corrected global cognitive composite score across the battery of tasks administered to the volunteers. Exploratory cognitive endpoints included baseline corrected scores from individual tasks. All right, let's go through the data. Eighteen volunteers developed infection by qPCR criteria of sustained viral load, one without

symptoms and the remainder with mild illness. Infected volunteers showed statistically lower baseline corrected global composite cognitive scores than uninfected volunteers.

Both acutely and during follow-up. They give us the mean difference over all the different time points with significant main effect of group and repeated measures. Sensitivity analysis replicated this cross-group difference after controlling for community upper respiratory tract infection, task learning, remdesivir treatment, baseline reference, and model structure. Memory and executive function tasks showed the largest between-group differences.

What happened here? In this study, the volunteers completed 11 computerized cognitive tasks in a fixed order on an iPad during two consecutive days pre-inoculations, we get a baseline each quarantine day and each follow-up visit. The 11 tasks included were motor control, object memory, simple reaction time, choice reaction time, 2D manipulations, this four towers, which looks at 3D visuospatial information.

Spatial span, which working memory capacity, target detection, which looks at attention and distractibility. A Tower of London, which measures spatial planning. Do verbal analogies, which measures semantic reasoning. Object memory, which measures the medium-term precision recognition memory. You can actually look at this figure where that everyone's sort of basically at the same baseline. Then you follow them out over time. You see that the infected have this really significantly lower cognitive score all the way out to a year.

VR: I don't understand why the score goes up for the uninfected from baseline. Why would that be?

DG: I think because you would expect when you repeat a test, people start to do better because they've done it before. In general - and it's really nice that they have a baseline here because you'd say, "Oh, they're just about the same." You're doing this task over and over, you should be learning, you should be getting better.

VR: I see.

DG: You could see like the uninfected people, they actually get better. They come up to this really nice and they hold pretty much there. After a while you can see like by the end of the year, they're getting a little tired of doing this. The infected people they don't really tend to like, learn over time. They found that the decrements in memory precision and executive function were the main contributors to reduced task scores following infection.

They found that volunteers who exhibited sustained viral load after inoculation with SARS-CoV-2 performed worse on a measure of global cognition than volunteers who did not exhibit the sustained viral load. This deficit persisted up to a year after inoculation. None of the volunteers reported subjective cognitive deficits, so they didn't even realize that they had all these deficits.

VR: I never thought this challenge study was a good idea because of this. How do you know this wasn't going to happen? You didn't. This is an unknown virus. This shows that it's not ethical. It was not ethical to do this. What do you think, Daniel?

DG: I share the same concern. Here they took these young, healthy volunteers, and these are good folk. These are folk, like altruistic. "I want to make a difference. What can I do?" Now they're cognitively impaired. They don't even realize it, but when you test them like this damaged their brains. Bad news. All right. No one is safe until everyone is safe. Even these folks. I hope everyone will pause the recording right here. Go to parasiteswithoutborders.com and click Donate. Every bit helps.

That's what allows us to do this work. We don't accept money from, what, big pharma and all these other sponsors. It's all you. It's all our listeners. We're now doing our Floating Doctors fundraiser. Ben LaBrot, the head of Floating Doctors, is going to be a guest on *TWiV* coming up soon. During the months of August, September, and October, we'll double your donations up to a potential maximum donation of $20,000.

VR: It's time for your questions for Daniel. You can send yours to daniel@microbe.tv. Ginny writes, "I received the excellent newsletters from Katelyn Jetelina, Your Local Epidemiologist." I don't know, Ginny. Maybe you shouldn't. “In the most recent one, she answered a question like this. "If my COVID antigen at home test is positive for a long time, 14, 21, 28 days, am I still contagious?" Here is the epidemiologist response. "Unfortunately, we don't have good data on this. Most likely you're still contagious." Wait a minute. She said they don't have good data, but she's making a conclusion.

DG: Yes.

VR: "COVID-19 antigen tests are very good at detecting infectious COVID-19 virus." Really? Really? "PCRs, conversely, are good at telling you whether you have the virus regardless of infectiousness." Oh my gosh. She's digging it deeper and deeper.

DG: Yes.

VR: "Be sure to wear a mask. Back to Ginny, to her credit, she writes, "Is that right? Since antigen tests detect protein, are they very good at detecting infectious virus? I thought you needed something like a plaque assay. (See, I've been paying attention.) Or is it that you won't get the protein unless the virus is active? I thought the antigen test might see remnants even after the virus is no longer active."

DG: OK. Ginny, thanks for writing us. This is a great chance for us to go through this. We do have good data. It's actually amazing. The data we have for COVID-19, for SARS-CoV-2 is actually better than the data we have on almost any other pathogen out there. It's amazing the amount of money and resources and studies that have gone into this. This is what we know. Unless you have significant immunocompromise, you get infected, you test positive, your symptoms start, that's day zero. After, we'll say, the first five days, 85% of the transmission events happen in those first five days.

We still see about 15% of the transmission in day six through 10. Really, it's day six, seven, maybe eight. We really don't even see much on day nine, 10. After day 10, we really do not see transmission, period. It does not correlate with symptoms. Folks that go on to develop that inflammatory phase, where they feel crummy, they do not become contagious again. We have studied this in, well, a billion people.

We do actually have really good data on when you transmit to others. The tests are great for making the diagnosis, but they're not great for tracking whether or not you're contagious. I still see to this day, it's day, let's say, 11, 12, 14, 21. People are testing to "be safe." If it's after day 10, you don't transmit to others. Those tests have no role in helping you make any further decision along those lines. Yes, Ginny, you are correct.

The antigen tests pick up the protein, they pick up the antigen, and they can remain positive for a very long period. The PCR also can remain positive for a very long period. It doesn't tell you about the virus, it tells you about the RNA, it detects RNA. If you want to actually pick up, is there a replication-competent virus? Yes, you need a plaque assay.

VR: When you need virological advice, you need to talk to a virologist, not an epidemiologist. Sorry. Mike writes, "Wrestlers and martial artists sometimes need to worry about things like ringworm and herpes gladiatorum. Do you think eventually we'd need to worry about mpox as well?"

DG: I'm not sure eventually. Wrestlers, there's a lot of skin-to-skin, there's a lot of close contact. Folks that have mpox are able to transmit from the time they start to feel sick. Even before you can actually see any skin lesions, they continue to transmit until they have full, intact skin in those areas. No, I think that we need to keep track because there may be some at-risk individuals participating in these activities, in the wrestling. Potentially it could get introduced into a population that way.

VR: Alec writes, “Greetings from Cambridge, Massachusetts. Hearing the letter about the mRNA vaccine disinformation in last week's clinical update motivated me to write you. My partner recently got COVID for the first time. She's 67 and healthy. We both had gotten Paxlovid prescriptions last winter with difficulty in advance of traveling, so she was able to start Paxlovid after her first positive test. After a couple of days, she's feeling much better. Our neighbors warned us, warned her about Paxlovid rebound.

Being a faithful listener of the *TWIV* clinical update, I said confidently that Paxlovid rebound is not a thing. She googled Paxlovid rebound, and the first result was a blog post for laypeople from a medical school in our area that you may have heard of, titled 'Paxlovid Rebound: What You Need to Know,' on the May 9th. It defines Paxlovid rebound, has sections, how to know if you're experiencing rebound, and managing rebound.

In short, it treats Paxlovid rebound as real. Buried in the ninth paragraph, it says, 'For people at high risk of getting very sick or dying from COVID, the benefits of Paxlovid far outweigh any risk of rebound.' It mentions the large clinical trials you often cite that show no evidence for Paxlovid rebound. The only evidence of rebound is a small observational study from Harvard Medical School.

The first sentence of the results section of this paper is, compared with untreated persons, (N=55), those taking N-R, (N=72), were older, received more COVID vaccinations, and more commonly had immunosuppression. I'm shocked that one of the most famous medical schools in the world would publish an article that might discourage people from taking a potentially life-saving treatment based on a concern that probably is not real. What is to be done?"

DG: Yes, this is tough and I worry about. A lot of times I even hear these "thought leaders, senior people," and you could tell that they're actually not reading the science. They're actually quoting from *The New York Times*. I'm like, seriously, if you're going to be the chief of a division, if you're going to be the one putting out this advice, you should actually have the integrity to read the science and not just recycle what sort of secondary information from the media. We're not here to be an echo chamber of the media. We're here to actually take the time and give reliable, credible advice.

VR: As Mark Crislip always said, "Read the literature."

DG: Yes.

VR: Carmen writes, "My son, now a 28-year-old New Yorker, was exposed to TB in South Africa at seven years old when his father took him for a prison visit as well as staying in the townships. He had a big new positive PPD four months later. He could not take INH because of bizarre hyper-behavior changes. He's been healthy since. If that TB exposure were to become a health issue for him, what ID team in New York City would do appropriate tracking of TB resistance to factor into treatment for him? I've been thinking to ask for a while."

DG: Yes. I think there's two aspects. One is that if there was this issue with INH, Rifampin would be another potential way of treating that latent TB because 5% lifetime risk of a relapse, one in 20. Columbia has a great department that would be happy to help.

VR: Connie writes, "Thanks again for your podcast. I recently read Jetelina's newsletter that it might be beneficial for people to get a pertussis vaccine every 10 years. She wrote, and referenced an article from the UK, that the pertussis vaccine that's been used for decades loses its effectiveness over time more so than expected. Can you give me any information? I'm 75, got a Tdap booster shortly before my oldest grandchild was born in 2016. Do I need another one in the near future?”

DG: Well, I guess everyone's reading Jetelina's newsletter. We do actually recommend the Tdap every 10 years. I'm not sure if I would say loses its effectiveness more than expected. What we also recommend is it's really the pertussis booster every pregnancy. This is something you want to do more than once in your life.

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

DG: Thank you. Everyone, be safe.

[END OF AUDIO]