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Case-Control Study of Individuals with Discrepant Nucleocapsid and Spike Protein SARS-CoV-2 IgG Results

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Guest: Dr. Hannah Wang is a medical microbiology fellow in the Pathology Department at Stanford University School of Medicine.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett. Over the course of the COVID-19 pandemic, substantial progress has been made in understanding the humoral response to infection with SARS-CoV-2 and antibody testing has come to play an important complementary role in COVID-19 diagnosis.

Antibody testing is used to evaluate patients with a high clinical suspicion of infection and repeatedly negative nucleic acid amplification tests, as well as in the assessment of suspected multi-system inflammatory syndrome in children.

SARS-CoV-2 antibody testing is also a critical public health tool enabling surveillance efforts to characterize seroprevalence and inform policy decisions.

Finally, SARS-CoV-2 antibody testing may be used to monitor the humoral response to vaccines. However, there is limited prospectively collected data on SA performance and minimal clinical information to guide interpretation of discrepant results.

A paper describing a case control study of individuals with discrepant nucleocapsid and spike protein antibody results appears in the July 2021 issue of *Clinical Chemistry* to help address that very topic. The lead author for that study is Dr. Hannah Wang. She is a medical microbiology fellow in the Pathology Department at Stanford University School of Medicine. She is board certified in Anatomic and Clinical Pathology and is interested in developing and evaluating rapid infectious disease diagnostics using novel molecular and computational tools. She is our guest in this podcast.

So, Dr. Wang, there have been several SARS-CoV-2 serology assay comparison study is done already. Some of them featured here in our podcast. Tell us why you wanted to conduct this study in the first place. What makes it unique?

Hannah Wang: Happy to. So, what makes the study unique is that this was actually driven by a clinical question that came up for us when

we were reviewing our results and what inspired the study really was that as a laboratory, we were switching from one assay to another.

So, we were switching from this anti-nucleocapsid assay to the anti-spike assay. And during that time, we have to validate both of the new tests and compare the results of the old versus the new test. And what we noticed was that there were a lot of discrepant results.

One patient would be positive on one assay and negative on the other. And that basically made us think, "Well, how are we going to be able to explain this to our physicians when they see their patient all of a sudden went from antibody positive to antibody negative or vice versa?"

The way we designed the study was actually as a case control study. So, we compared all of the samples that came into our lab over a two-week period and we ran them on both assays. And this is a little different than how typically an assay comparison study would be done where you would kind of select some chosen positive samples and some chosen negative samples and compare them. What we were looking at was everybody that any clinician had ordered antibody testing on and we took all comers for the past two weeks.

So, I think what makes our study unique is that the patient population that we were looking at in this study was the exact same patient population that we would be using the test on. And that's not always the case when it comes to assay comparison studies in the laboratory.

Bob Barrett: What surprised you the most about the results?

Hannah Wang: What was surprising was that there were a lot more discrepant results than we expected. There are actually more results that were discrepant between the assays than were positive on both assays.

Bob Barrett: Why do you think you found discrepant results more frequently than the prior studies that compare the Abbot anti-Nucleocapsid IgG and EUROIMMUN anti-spike IgG assays?

Hannah Wang: So, I think this has a lot to do with our study design where we did not choose kind of well-characterized positives and negatives. We were looking at basically all of the gray zone cases where clinicians were ordering testing and these included patients who were maybe further out, months out from their initial infection or individuals who were only a few days out from their original diagnosis. And these cases usually wouldn't be used in an assay comparison study because they do tend to be in a gray zone.

Bob Barrett: So, what should laboratorians, clinicians and patients do when they're faced with discordant serologic test results?

Hannah Wang: I think this is a great question and I think part of the answer lies in the laboratory and part of the answer lies on the clinical side of things.

So, when you're faced with a discrepant result, I think it's important to first understand: what are the two assays that are being done? And in our case, this IgG nucleocapsid versus IgG spike assay, they were two different antigen targets. One to nucleocapsid and one to spike. Now, this study was done before vaccines are widely available.

But you can imagine now that people have been vaccinated, you might get more frequent discrepant results based on this antigen target. A lot of these, especially the MRNA vaccines, are targeted against spike. So, if you have a discrepant result, that may be the result of being immunized, for example, against spike versus nucleocapsid.

The other difference in laboratory assays is the type of assay being done. And so, one of these assays was an ELISA and one of these assays was chemiluminescent amino assay. So, a different method basically. And those small differences in methods can also affect outcomes and results and cause discrepancies. So, that's in the lab.

And then the other part that we have to look for this answer in discrepancies is in the clinical side of things. Perhaps, a line of period has elapsed between the first test and the second test that you may have switched from negative to positive or positive to negative. Or perhaps, this individual was immunosuppressed in between and isn't producing as many antibodies anymore or potentially somebody was immunized or got COVID in between the two tests. So, those are kind of all of the factors that has to go into considering why two assays may be escorted.

Bob Barrett: Doctor, do you believe that there is any role for reporting quantitative or semi-quantitative serologic test results? And if so, what could that be?

Hannah Wang: That's a fantastic question. So, what we noticed in the study was that individuals who tended to be on the higher end have more antibodies tended to be positive by both assays. And those individuals that were really on the low positive end or borderline end, those were the ones that were not reproducible.

So, I do think there may be a role for some sort of semi-quantitative reporting of results or potentially dual testing or

reflex testing by two different methods to get a sense of how reliably positive somebody may be.

So, one example of this is at Stanford, we actually reflexed all positive antibody assays to a blocking assay. And what our blocking assay does is it doesn't just assess the quantity of antibody present but also the quality and the ability of the antibody to block binding between receptor binding domain and the ACE-2 receptor.

Bob Barrett: Finally, Dr. Wang, what do you see for the future in SARS-CoV-2 serologic testing as more and more of the population is getting vaccinated?

Hannah Wang: So, I think there is a future for serologic testing as a way to distinguish individuals who have been immunized from those who have not. But I do think that it's important to keep in mind that these assays were not designed with that specific goal in mind. And that's something that we discovered on this assay is that it's possible to be spike positive but nucleocapsid negative, just having been somebody who actually got COVID.

So, just being spike positive and nucleocapsid negative isn't necessarily an indication, based on these assays, of somebody being immunized. And so, I think we have to be careful about the interpretation of these assays before we have real validation data in individuals who are immunized versus those who are derived natural immunity.

Bob Barrett: That was Dr. Hannah Wang from the Department of Pathology at the Stanford University School of Medicine in Stanford, California. She has been our guest in this podcast on SARS-CoV-2 immunoglobulin assay comparisons. She is lead author of the paper describing that study that appears in the July 2021 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.