Clinical Chemistry

Associating SARS-CoV-2 Serological Assays with Protection: Where the Field Stands

Article:
Mei San Tang, Christopher W. Farnsworth.
Associating SARS-CoV-2 Serological Assays with Protection: Where the Field Stands.
Clin Chem 2021; 67:5 707-709. https://doi.org/10.1093/clinchem/hvab039
Guest: Dr. Christopher Farnsworth is an assistant professor in the Department of Pathology and Immunology at Washington University in St. Louis and the medical director of clinical chemistry and point of care testing and Barnes Jewish Hospital. Dr. Mei San Tang is a 3rd year and chief resident of Clinical Pathology at the Washington University in St Louis.

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. The emergence of severe acute respiratory syndrome coronavirus 2 or SARS-CoV-2 in 2019 was met with a rapid introduction of commercially available serological assays for SARS-CoV-2 antibody detection. Soon after being declared an emergency in the U.S., hundreds of serological assays for SARS-Cov-2 were introduced. Some of dubious quality exceeding the total number of assays available for any other infectious disease, and, in fact, most laboratory analytes. Even as laboratories gained access to this unprecedented number of assays, the utility of SARS-Cov-2 serological testing remains unclear.

However, a study published in the May issue of *Clinical Chemistry* has begun to address this important clinical question. That paper evaluated high-throughput SARS-Cov-2 serological assays in terms of clinical sensitivity, clinical specificity and association with virus neutralization tests. That paper was also the subject of an editorial in the same issue of *Clinical Chemistry*, titled "Associating SARS-Cov-2 Serological One Assays with Protection", where the field stands. That editorial was authored by Dr. Mei San Tang and Dr. Christopher Farnsworth from the Department of Pathology & Immunology at Washington University in St. Louis, and they are both our guests in this podcast.

So, Dr. Tang, let's start with you. At the time of this recording, we're about at month 16 of the COVID-19 pandemic in the United States, and we have learned quite a bit about immunity conferred from prior SARS-Cov-2 infection. Can you tell us what we currently know, and what we don't know about the impact of prior infection on future immunity?

Mei San Tang: So, in general, infections with common respiratory viruses, including the seasonal coronavirus would lead to production of neutralizing antibodies. These antibodies have the specific function of inhibiting viral

entry into cells within the infected individual, and they are therefore protective against reinfection. So, this is also the reason why there was so much focus on antibody testing at the beginning of the pandemic. But much of the unknown at the beginning was also whether immunity against SARS-Cov-2 would similarly involve neutralizing antibodies.

Now, after more than a year in the pandemic, we know that there is some evidence that patients who developed an antibody response from a previous infection may be protected from future reinfections. For example, there have been two large multicenter cohort studies from the UK reporting that individuals who had detectable antibodies were significantly less likely to develop an infection over a 6-month follow-up period. There are some concerns that natural immunity for SARS-Cov-2 may not be long-lasting since the levels of detected antibodies do decay over time in many individuals. Decreasing antibodies as to infection is clearer as a phenomenon commonly seen with many viral infections. Even though antibody levels may decrease, memory B cells persist and can rapidly increase in case of a reinfection.

So, for example, a recent study showed that plasma blasts that specifically produce SARS-Cov-2 antibody can be found in the bone marrow of individuals who were previously infected up to almost a year after mild infections. These are long living cells that reside in the bone marrow and were able to generate a robust immune response when challenged experimentally with SARS-Cov-2 protein. While this was a small study that looked at only less than 20 patients, the presence of these cells provides some evidence that there is durable memory response to SARS-Cov-2 and could potentially protect from reinfection and would be likely to help limit severe symptomatic infections.

Bob Barrett: Dr. Farnsworth, you and Dr. Tang wrote this editorial in response by the work Antonin Bal and his colleagues in France that was published in the same issue of *Clinical Chemistry*. How does their work add to the growing body of literature?

Christopher Farnsworth: Yes. So, as Dr. Tang pointed out, one of the body's primary means of protection from future infection is the production of neutralizing antibodies. So, there's really been a lot of interest from both a research perspective and clinically, and knowing on what concentration of neutralizing antibodies are present after an infection, and how long they last for. And unfortunately, the gold standard of this is called viral

neutralization assays. And what you do, is you incubate live SARS-Cov-2 virus with human plasma and then you take these, and you incubate them on live cells in culture, and you want to observe the limitation of these cytopathic effects. Basically, increased neutralizing antibodies will result in decreased cytotoxicity to the cells, in vitro.

And I say that unfortunately, because this gold standard method requires enhanced safety protection, so biosafety level 3 facilities, which they're really limited to large academic institutions.

And even at large institutions there's really only usually one, and there's only a couple of investigators that have access to them, and the throughput of these assays is just really low. So, there's been a lot of interest in trying to correlate these neutralizing titers with results from commercial antibody assays. So, the question is, can we use these commercial antibody assays as a surrogate for neutralizing potential. The commercial assays rather, are a bit different that they detect both neutralizing antibodies and what we called binding antibodies. These binding antibodies may still have a protective role, but in and of themselves, do not provide protection. And what this manuscript really demonstrates is that there's generally a modest correlation between neutralizing antibody titers and the signal produced by commercial assays.

So, if someone has really low neutralizing antibodies, they're likely to have low results by commercial assays. Hence, and if they have high neutralizing antibodies, they're are likely to be high by commercial assays. However, the relationship is far from perfect. So, another important finding is that there's actually low concordance between the two with .72 being the highest concordance between the nine commercial assays that they tested and the neutralizing assay that they used. So, what this implies, most importantly, is that there's numerous patients that potentially have antibodies present by commercial assay, but are below the limit of detection by neutralizing antibody assays.

So, these kind of argue against the use of serologic antibody results from these commercial tests as evidenced of viral neutralizing capability, and really argues against the use of these assays, the commercial assays for demonstrating the degree in length of protection. And actually, this is in step with the recent release from the FDA, who's really advised against the use of serologic testing, even quantitative

assays, for determining the likelihood of future protection from SARS-Cov-2. And this is due at least in part because we don't know what the protective titer is or what result will be from each of the commercial assays and how it correlates to neutralizing antibodies.

Bob Barrett: You mentioned in your editorial that the immunity provided from previous SARS-Cov-2 infection maybe mediated by cellular immune responses, and the lack of correlation with neutralizing antibody assays and commercial serologic assays does not mean that someone may or may not be protected. Can you tell us why this may be Dr. Tang?

Mei San Tang: Yes. So, the commercial serologic and neutralizing antibody assays that we've been discussing so far can only measure antibodies that are circulating in our blood. However, it's also important to remember that an effective immune response against viral infections can also involve other cell types that do not directly produce antibodies, but instead exert an antiviral effect, either by directly producing substances that are toxic to the infected cells or help amplify antibody production. This is known as the cellular immune response and does not always correlate with the antibody mediator immune responses. And we know from some studies that some individuals, especially those who had milder infection symptoms, appear to have a weaker antibody response, and instead would mount a stronger cellular immune response. So, they're not always concordant, and the important take-home point here is that a weak or undetectable antibody response measured by these commercial serologic assays after natural infection may not necessarily always indicate that the individual will not be protected from reinfection.

Bob Barrett: Well, finally, Dr. Farnsworth, what do you see as the main role of serological testing for patients with regards to vaccination or vaccination status?

Christopher Farnsworth: It's very interesting to see how things have changed throughout the COVID-19 pandemic. So, you know, right now we're in July of 2021 or just about July 2021, and this was originally written, the draft of this editorial was in February, and those intervening months we went from almost none of the U.S. population vaccinated to COVID-19 to now over 50% of adults. And that intervening time, there's really been a lot of calls for how we should use serology for vaccination. And one of the things that people have said, is that maybe we can use serology to prior to

vaccination to see if someone was previously infected, could we conserve vaccine by perhaps only giving them one dose as opposed to two doses for a lot of the mRNA vaccines.

The primary problem with this approach is that there's actually no data that compares the efficacy in these two populations. So indeed, if you have one -- if you had a previous infection and then you get one dose of vaccine, you tend to have a similar serologic response to someone that hasn't been infected and got two doses. But it doesn't necessarily mean that they've been conferred equal protection, and no clinical study has actually shown this in a randomized control trial that it's equally effective.

So, as Dr. Tang pointed out antibody responses are only part of the story when it comes to protection, so they use commercial assays as a surrogate for infectious outcome. That's currently inappropriate. Similarly, operationalizing this approach is quite daunting. So much like the issues they had with implementing mass testing for diagnosing SARS-Cov-2. There's so many preanalytical issues with trying to perform phlebotomy, to accessioning all these samples, registering them, delivering the results, trying to get that to whole countries of individuals, is just a daunting task.

So, that's kind of one of the ways that people have proposed that we should use commercial assays. The second has been that we should test at-risk groups after vaccination to confirm that they had a sufficient immune response. And the data here on a research front is actually quite compelling. So, patients, for example, with cancer that are currently under treatment, hemodialysis and other comorbidities simply do not mount the same serologic response after vaccine in many cases as healthy control populations.

So, from a patient perspective, the desire is to know if the vaccine actually conferred protection. And it seems very simple and straightforward and important with the hope that it can inform, "Can I safely unmask? Can I travel on airplanes? Can I hug my child?" The problem though is that there's no cutoff available by any of these commercial assays that currently tells us protection. So, if someone is below the threshold for a positive result by one of the commercial assays, it doesn't mean that they have no protection. Again, as Dr. Tang pointed out, lots of cellular responses that could have been involved in that protective response. And I think even more

importantly, if they have a high result, there's not data to imply that they actually have more protection.

So furthermore, if someone is low, there's no clear indication as to what we should do clinically. So right now, there's no evidence that we should revaccinate these individuals or give them a third, or even fourth dose. So really, what we can tell them is, to just use broad safety precautions like they have been: like continue masking in public areas, hand-washing, social distancing, which frankly is probably good practice for people who have some pretty serious comorbidities, like active cancer on treatment, et cetera. This is, again, pretty much in line with what the FDA has recommended. We are not operating in a vacuum. They said the antibody testing should not be used on vaccinated patients prior to or following administering a vaccine. And the primary risk here is the potential that people will take fewer precautions when they really should not be taking those precautions just based on a result where we don't know what's actually associated with protection.

I think the last thing I'll kind of leave us with, is that even though it may not be useful in like a clinical setting. From a research perspective, these commercial assays have been tremendous and they're really helping us to understand how we respond serologically after SARS-Cov-2 infection. You know, really being able to monitor the vaccine response to these various populations has been really interesting, and probably data that continues to come forward and the rest of the year will help inform us as we go into the fall as to "How should we manage patients with various comorbidities?"

Bob Barrett: That was Dr. Christopher Farnsworth from Washington University in St. Louis. He was joined by his colleague, Dr. Mei San Tang. They have been our guest in this podcast on, where the field stands on associating SARS-Cov-2 serological assays with protection. Their editorial and the original scientific paper examining SARS-Cov-2 serological assays appear in the May 2021 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.