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TOP-Plus is a Versatile Biosensor Platform for Monitoring SARS-CoV-2 Antibody Durability

Article:
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Guest: Dr. Zhen Zhao is an Associate Professor of Clinical Pathology and Laboratory Medicine and the director of the Central Laboratory and Clinical Chemistry Service at Weill Cornell Medicine in New York City. Dr. Sabrina Racine-Brzostek is an Assistant Professor at Weill Cornell Medicine's Department of Pathology and Assistant Medical Director of Central Laboratories.

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.

Coronavirus disease 2019 is led to crippling levels of morbidity and mortality around the world. Seroprevalence studies initially indicated a larger extent of SARS-CoV-2 infections than initially reported because of the high prevalence of infected individuals with mild or no symptoms. However, not all such studies examined antibody avidity which may be important in assessing long-term immunity. A paper appearing in the September 2021 issue of *Clinical Chemistry* evaluated both the level and the avidity of SARS CoV-2 receptor binding domain antibodies using a testing on a probe approach that includes a newly-developed avidity assay. The senior author of that study is Dr. Zhen Zhao, an Associate Professor of Clinical Pathology and Laboratory Medicine and the director of the Central Laboratory and Clinical Chemistry Service at Weill Cornell Medicine in New York City. She is joined in this podcast by her colleague at Weill Cornell and lead author of that paper, Dr. Sabrina Racine-Brzostek, and we'll start with you Dr Zhao.

Can you briefly tell us what is avidity and what role it plays in immunity?

Zhen Zhao: Sure. Before talking about avidity, I would like to introduce a term called "affinity". Affinity is the strength of a single bond or interaction in terms of the antibody/antigen relationship. The binding affinity is the strength of the interaction between the antigen's epitope and the antibody's paratope at a singular binding site. You can take a step further by defining "avidity". Avidity is the term used in immunology to describe the total binding strength between an antibody and its corresponding antigen, because most interactions are multivalent, and you take into account all of the individual interactions. That is why avidity is sometimes called "functional affinity". Affinity and avidity are both measures of binding strength.

Bob Barrett: Dr. Racine-Brzostek, I'd like to bring you in now. How does antibody avidity play a role in COVID-19 natural immunity?

Sabrina Brzostek: Yeah. That's a great question. And in order for us to start answering that question, we first need to talk about viral infections in general, and try to get a better understanding of antibody affinity and avidity post-infection. So typically, after the initial introduction of the virus to the host along with the subsequent early antibody response to that virus, you will find that affinity is low. Meaning that although there is binding, it may be weak. This low affinity is seen despite the large initial increase in overall total antibodies against the pathogen. Now over time, even though the overall total antibody level may decrease, the avidity actually strengthens and matures. When you look back at studies focusing on the first SARS outbreak, the SARS CoV-1, they saw just that. Low antibody avidity early in the infection and this avidity matured and continue to increase within the first month of symptom onset. This maturation is seen in all other types of infections such as EBV and CMV.

So why is this? Well with maturation, the intrinsic affinity of the antibody antigen interaction strengthens, and so does that functional affinity of the bivalant IGG that's being produced. The reason for this is that avidity maturation is based on the proliferation of the B cells that produce the IGG, as well as that ever-continuing hyper mutation of that variable part of the immunoglobulin genes. The B cells can also undergo clonal selection by selecting those B cells that express IGG of higher affinity on the surface compared to those of the ones that are expressing lower affinity. And so, as more research is being done on avidity in COVID-19, we see a similar pattern emerging with SARS CoV-2 as well. Initially, avidity could be low but with time, in most populations at least, you'll see it strengthening.

Bob Barrett: So, can you tell us about your assay and how it measures avidity?

Zhen Zhao: Sure. We have previously published testing on the probe TOP biosensor to quantify SARS CoV-2 total antibodies and neutralizing antibody activities. Our collaborators at ET Healthcare modified the TOP biosensor; we then called the new sensor TOP-Plus for the measurement of antigen/antibody binding kinetics. It may actually be helpful for audience to take a look at our Figure 1 in the paper, especially if you are not familiar with binding kinetics.

Briefly, SARS CoV-2 antibodies are measured at the tip of a receptor binding domain, RBD, coded QUAL probe, and we used about attenuated RBD and Cy5 streptavidin conjugate as signaling elements by calculating the relative dissociation

rate, we were able to define avidity in this new assay. This dissociation profile represents the rate of antibody dissociation from the RBD coded probe which can be a measurement of avidity. A lower relative dissociation rate reflects both affinity maturation and multivalent binding development, and hence avidity maturation.

Sabrina Brzostek: Yeah, and so Dr. Zhao has done a great job in describing how our assay works. I'd like to add some additional advantages to our biosensor. So, our assay is not the first to measure antibody/antigen interactions, and antibody avidity could be measured in a variety of ways. You could use methodologies such as ELISA HPLC, Capillary Electrophoresis, or even single radial immunodiffusion, and then try to measure the disruption of the antibody/antigen interaction one form or other. These methodologies often just give you an overall qualitative picture. They're labor intensive, are low throughput, and in some situations, you can argue these methodologies could display low accuracy and precision. Therefore, biosensor technologies such as surface plasmon resonance and biolayer interferometry have become popular in monitoring molecular binding between the antigen and antibody in the more real time and cost-effective manner.

However, the most common approach in these methodologies in assessing avidity is the disruption of that antibody/antigen binding with some sort of chaotropic agents such as urea. By using this technique, you can argue that the assessed avidity of the antibody in that particular setting depends on its resistance to the chaotropic agent and may not truly represent the avidity of antibody towards the antigen. So, our TOP-Plus avidity assay that's presented in the Clin-Chem paper distinguishes itself from others in that it does not apply to chaotropic reagent. Therefore, the measured relative disassociation rate better reflects the natural disassociation rate of antibodies from the target antigen than in the other approaches, where the chaotropic agent may actually alter that native structure of the antigen or antibody.

Bob Barrett: Doctors, tells us what else makes this particular assay and its use so interesting?

Zhen Zhao: So, remember how we talked about avidity maturation. Well by looking at both, the total antibody levels and the avidity, you can get a better idea about the age of the immune response. And on neutralizing activity, you have a great even profile, at least in terms of the antibody response. In this Clin-Chem manuscript, we demonstrated this use. We evaluated the antibody response and antibody avidity about one-month and six-months post on symptom onset in 80 individuals previously diagnosed with COVID-19 and saw that the avidity increased while observing decay in a total SARS CoV-2 antibodies and neutralization antibody activity. We

also demonstrate in the paper, which at the time was worked early on in the vaccination campaign, that antibody avidity in turn vaccinated individuals about one month, post the first dose of SARS CoV-2 vaccination had similar avidities to those that had been exposed to the virus during a similar time period.

Sabrina Brzostek: Yeah, and now as we all know COVID-19 a field that's quickly evolving, and one of the things our public health agencies need are better tools to better help answer their questions about SARS CoV-2 immunity. For instance, do the antibodies generated from the prior COVID-19 infections help protect against the various variants that arising all over the world? I mean, just recently both Lambda and Mu were named as variants of interest by the WHO. If you are able to quickly swap out that wild type RBD in our assay for the RBD of some other variants, you can now potentially start looking at an individual's or even the overall population's immune response against the various variants of SARS CoV-2.

Bob Barrett: Well, finally doctors, is there any potential application for this on a larger clinical scale?

Zhen Zhao: Avidity assays can be good supplemental diagnostic tools in battling diseases. If you were to go search PubMed, you would find users in diagnostic medicine to help identify or exclude pathogens like toxoplasma, primary CMV, and acute rubella virus infections. It has also been used for aiding in a diagnosis of ABV, HIV, West Nile virus, and the previous SARS CoV-1 infections. It could also be used in the development of new candidate pharmaceuticals, where avidity can be an indicator of how well the pharmaceutical interacts with its target, giving the researchers an idea of how effective it could be at treating certain diseases, as well as similar applications in oncology research when it comes to anti-cancer therapeutics and tumor targeting. You can look at this past avidity applications to see how it could be used for COVID-19.

Sabrina Brzostek: Yeah, absolutely, Dr. Zhao. It's hard to believe, but it's only been only about 18 months since SARS CoV-2 first hit the U.S. So early on, anyone testing positive on serology for COVID-19 was known to have a "recent infection". As time goes on though, it's going to become more and more difficult to distinguish recent versus past infections, and therefore, more difficult to do any type of tracing. So, for instance was "Mr. Smith's positive serological testing reflective of an old asymptomatic infection from 2020," or is this a positive serology in an asymptomatic infection from a week or two ago? Also around this time, his daughter may have had a cold or allergy that ended up being COVID-19. Did she get it from a classmate at school or from someone at home such as Mr. Smith?

So, antibody levels alone may not necessarily answer that question. With avidity testing, you can start grading the reaction; low avidity towards SARS CoV-2 would be a strong indication for an acute infection and you could further monitor to watch the subsequent avidity maturation as a confirmation of that. A higher avidity against SARS CoV-2 would indicate a remote past infection. Again, all of this of course would need to be validated for clinical use, but with all the cases we're seeing out there these days, it would be plausible. Furthermore, this particular platform the TOP-Plus would also give you the additional information to help with that overall evaluation. It includes levels of IGG, IGM, the total antibody, as well as neutralization activity of those antibodies. Together with the avidity, you would have a very good idea of the patient's overall antibody response to the virus, especially if you could swap out the RBD to the variant RBT of interest. It has useful applications in the research setting as well as the clinical and public health settings.

Zhen Zhao: Up to now, Dr. Racine and I have been discussing the antibody response to the virus but you can apply the same concept to vaccine development. This is a big question right now. Back there, our vaccination programs are effective in certain patient populations like the immune compromised. You can look at the IGG or the total antibody production and try to correlate that with vaccine efficacy, but there are other parameters that are helpful and can give you a better overall picture neutralizing activities and avidity could also be monitored with outcome measures by looking at total antibodies, neutralizing antibodies and avidity together, we may be able to gather a better idea of an ideal vaccine induced immune responses against SARS CoV-2 and all of the variants of concern. With this data, we can make better data-driven decisions on when interventions such as booster shots are needed, and in which population.

Bob Barrett: That was Dr. Zhen Zhao, joined earlier by her co-author, Dr. Sabrina Racine-Brzostek in this podcast on monitoring SARS CoV-2 antibody durability. They're both from the Department of Clinical Pathology and Laboratory Medicine at Weill Cornell Medicine in New York City. Their paper appears in the September 2021 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.