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Editorial

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

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The emergence of severe acute respiratory syndrome coronavirus 2 (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 US, hundreds of serological assays for SARS-CoV-2 were introduced, exceeding the number of assays available for any other infectious disease and most other laboratory analytes. As a result of this rapid expansion and at times dubious quality, the Food and Drug Administration (FDA) began reviewing and regulating all SARS-CoV-2 serological assays under emergency use authorization (EUA). To date, 55 serological assays have received EUA. Nonetheless, as laboratories gained access to this unprecedented number of assays, the utility of SARS-CoV-2 serological testing remained unclear.

Despite questions regarding clinical utility, many hospital laboratories opted to implement SARS-CoV-2 serological assays. One of the most common uses observed has been among the curious-well, a subset of healthy individuals who experienced mild or no symptoms consistent with SARS-CoV-2 infection but were inquiring about their serological status as outpatients. This is likely due, at least in part, to the much-promoted idea of an "immunity passport", which presumes that an individual with antibody is no longer susceptible to SARS-CoV-2 infection. Assay manufacturers and academics continued to promote this idea, largely in response to the persistent lockdown strategies and an increasingly frustrated general public (1). However, for the vast majority of 2020, there was sparse evidence that prior SARS-CoV-2 infection conferred protection from reinfection.

One year since from the start of the pandemic, there is now clinical evidence that infection with SARS-CoV-2 does impart some protection from reinfection. A study in the United Kingdom demonstrated that healthcare workers with antibodies to the SARS-CoV-2 spike protein were ∼10x less likely to be infected in the ensuing 6 months than those without antibodies (2). The prevailing scientific rationale for presumptive immunity based on antibody testing is antibody-mediated viral neutralization, where host lymphocytes produce neutralizing antibodies that inhibit viral entry into host cells, thereby preventing infection. In an outbreak among a fishing boat crew, 3 crew members who had developed high concentrations of neutralizing antibodies prior to departure did not develop reinfection despite the high infection rate, suggesting that neutralizing antibodies may have a protective effect against SARS-CoV-2 (3). Furthermore, studies with convalescent plasma have demonstrated improved outcomes, including reduced mortality, in patients receiving units of plasma with high antibody titers relative to those receiving units with low titers (4). Together, these results are promising that patients with antibodies to SARS-CoV-2 have some protection from subsequent reinfection, although the durability of presumed immunity is still relatively unknown (5).

In contrast to the ease of measuring total anti-SARS-CoV-2 antibodies, measuring neutralizing antibodies has required highly laborious assays that are limited to research use. Neutralizing assays involve incubating patient plasma/serum at different dilutions with live virus. This is then inoculated into cell lines to observe for cytopathic effects. Neutralizing titer is typically reported as the dilution required to inhibit cytopathic effects by 50%. Due to the use of live virus, neutralizing assays are performed in biosafety facilities and are limited to research institutions. While some assays have utilized a pseudo-virus approach (most frequently Vesicular Stomatitis Viruses engineered to express a portion of the SARS-CoV-2 viral spike protein) and have reported concordant performance with neutralizing assays, one attractive alternative is to use commercially available serological assays to predict the presence of neutralizing antibodies. To this end, studies performed in hospitalized patients with severe infection have found modest correlation and poor agreement between SARS-CoV-2 serological and neutralizing antibody assays (6). However, little to date has been published associating neutralizing titers with commercial serological assays in mildly symptomatic and asymptomatic patients; populations with typically less

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pronounced immune responses including antibody concentrations (7).

A study published in this issue of *Clinical Chemistry* by Bal et al. has begun to address this important clinical question (8). The authors collected 439 longitudinal plasma specimens from 76 healthcare workers with mild, polymerase chain reaction (PCR)-confirmed SARS-CoV-2 infection, and 104 specimens from 44 patients with severe infection requiring ICU admission. They then tested each specimen by 9 commercially available SARS-CoV-2 serological assays and a neutralizing antibody assay. The authors made several important observations. Among these, the most important finding is 6-fold lower neutralizing titers in patients with mild infection relative to patients with severe infection. While outcome studies are required, this suggests that patients with mild symptoms or asymptomatic SARS-CoV-2 infection may have limited protection from future infection relative to those with severe infection, and the durability of that protection could be reduced. Another important finding from this study is the relatively low concordance between commercial assays assessed and neutralizing titers, with 0.72 as the highest concordance of the 9 assays. This implies that numerous patients had antibodies present by commercial assay but were below the limit of detection (1:20 titer) of the neutralizing assay. These results argue against the use of serologic antibody results from commercial assays as evidence of viral neutralizing capacity, regardless of the viral epitope detected by the assay.

The authors should be applauded for both the breadth and depth of their study, which adds to a growing body of literature cautioning against the use of commercial serologic assays for distinguishing future protection. However, a few caveats should be noted when correlating serological assays with neutralizing titers. First, immunity to SARS-CoV-2 can be mediated by cellular immune responses and a lack of correlation with neutralizing antibody assay does not necessarily preclude using seropositivity as an indicator of immunity. SARS-CoV-2 IgG concentration has been shown to correlate with SARS-CoV-2 specific T cells (9), while in vitro neutralizing assays do not necessarily reflect T cell-mediated immunity and can be discordant, particularly in mild SARS-CoV-2 infection (10). Furthermore, SARS-CoV-2 memory B cells appear to persist even as antibody concentrations reduce over time (11). With regards to neutralizing antibodies, while the authors found good agreement between neutralizing titers of 1:20 and most commercial assays, the overall agreement dropped considerably if the cutoff for a positive neutralizing titer was raised to 1:80. Notably, both the FDA and early vaccine trials have implied protection at neutralizing titers >1:250 (4, 12), and previous studies have found a negative percent agreement of <40% between commercial assays and neutralizing titers >1:256 (6). In short, despite the burgeoning literature about SARS-CoV-2 serology, more studies are needed to identify protective antibody concentrations and durability of protection from reinfection before commercial assays are useful for this purpose.

While high throughput serologic assays may not yet be an appropriate tool for determining protection from SARS-CoV-2 reinfection, this does not imply that they have no role clinically. Serological testing may be useful for diagnosis of multisystem inflammatory syndrome in children, diagnosis in symptomatic patients who present >14 days from symptoms and are persistently SARS-CoV-2 PCR negative, and for identifying convalescent plasma donors. To this end, the current standard for convalescent plasma is to label a unit as "high titer" if the donor is tested to have an assay signal of ≥9.5 on the Ortho Vitros Anti-SARS-CoV-2 IgG assay. A signal of 12 on this assay was reported to correlate with a titer of 1:250 on a neutralizing assay performed at The Broad Institute (4). Importantly, "high titer" convalescent plasma units were associated with improved outcomes when administered early (13). While the minimum neutralizing titer required for therapeutic effect has not yet been established, it is also unclear what quality control materials are available for precision studies at this high titer signal. Nonetheless, the Ortho assay and cutoff of 9.5 will be implemented at blood centers across the US for identification of high titer units.

Finally, will there be any role for serological testing as vaccines for SARS-CoV-2 become available? Clinically, this is still not immediately clear. Some have proposed the use of serological testing to prioritize vaccine allocation. However, this study adds to the growing body of literature that seropositivity does not imply robust protection in mild cases of COVID-19 (8). The CDC states that those with documented acute infection in the previous 90 days may choose to delay vaccination to allow others to be vaccinated, mainly because few cases of reinfection within 90 days have been documented. However, previous infection is not considered a contraindication and the CDC recommends *against* prevaccination serologic testing (14). Furthermore, serologic testing following any current routine vaccinations is not standard clinical practice. Using serology results to manage vaccinations is usually limited to specific situations such as the evaluation of an incomplete vaccination record to decide if additional vaccines should be administered (15) or determining the need for booster dose in special clinical situations such as pretransplant or postexposure prophylaxis. Even in these situations, only a handful of vaccine-preventable diseases have serologic assays that can be used for such purposes. Nonetheless, well-validated quantitative SARS-CoV-2 serological assays can serve a role in research studies to

establish protective titer following vaccination. Given the low throughput and high cost of neutralizing assays, it will be important for future studies to assess correlates of protection from vaccination using higher throughput assays. Finally, it is important to note that as vaccines are ubiquitously administered, serologic assays that target anti-spike antibodies will no longer be useful for identifying natural infection. This may have important ramifications for ongoing clinical studies assessing the prevalence of SARS-CoV-2 infection and when using serology to aid in diagnosis.

Where do we stand with correlating commercial serological assays for SARS-CoV-2 with protection? The work of Bal et al. certainly brings us closer to understanding the role of serologic testing for this purpose. However, more questions remain to be answered in the coming months, particularly in the context of vaccination.

Nonstandard Abbreviations: EUA, emergency use authorization; FDA, Food and Drug Administration; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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