Role of serology in the COVID-19 pandemic

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In this issue of Clinical Infections Diseases, F Xiang et al present a serologic study of 85 nucleic acid test (NAT) SARS-CoV-2 positive patients and 24 NAT negative patients who had symptoms suspicious for COVID-19[1]. Sixty controls were also evaluated and included healthy healthcare staff and patients with a variety of diseases from bacterial pneumonia to lung cancer. Serological testing consisted of an enzyme linked immunosorbent assay designed to detect IgM and IgG antibodies against the N protein of SARS-CoV-2. Serial examination of COVID-19 patients resulted in IgM seroreactivity by day 4 post symptom onset, which peaked by day 9. In contrast, IgG sharply increased 12 days after symptom onset; all NAT positive patients were positive for IgG 30 days post symptom onset. In patients with suspected COVID-19 who tested NAT negative, IgM antibodies were detected in 87.5% and IgG was present in 70.8%. In contrast, only 3 individuals in the control group tested positive for IgG but IgM positivity was not observed. The authors calculated a sensitivity for diagnosis of COVID-19 using IgM of 77.3% and a specificity of 100% while for IgG the sensitivity was higher at 83.3% and the specificity was 95%.

This article highlights a fundamental challenge clinicians face when patients present with COVID-19 symptoms yet are NAT negative, sometimes even with repeated NAT testing. As laboratory medicine physicians, we understand this dilemma and know that preanalytical variables can frequently underlie inaccurate results for all tests. In the case of COVID-19 NAT, preanalytical variables can include, but are not limited to, inconsistency in obtaining nasopharyngeal swabs, the different swabs and transport media used, time and temperature of specimen transport, and possible inhibitors to nucleic acid detection in the sample. In addition, there are likely analytical variables that can be explained by inherent differences in the ability of various NAT platforms to detect SARS-CoV-2. Importantly, a lack of understanding regarding the frequency and variability of viral shedding in patients with COVID-19 also makes it difficult to decipher how these variables may also contribute to false negative results.

Although NAT diagnostic testing continues to become a common approach to diagnosing infectious diseases in general, serology preceded NAT testing by many decades and still serves as the primary diagnostic tool for many diseases, including HIV, hepatitis B and C. This is in part due to cost and because NAT testing remains less sensitive for some pathogens, as occurs in syphilis. Different strategies are used for diagnosis by serological methods, for example, in leptospirosis a fourfold increase in IgG in convalescent versus acute serum frequently helps determine the diagnosis. In contrast, to diagnose dengue or American trypanosomiasis, NAT is most likely to be informative during acute disease while serology is helpful at later stages. In the case of the 2003-04 SARS-CoV outbreak, serology was primarily an epidemiological tool that could help determine the number of silent infections, how the disease progressed, defining transmission patterns, and the possible origin of the virus itself[2].

In the case of COVID-19, while serological analysis may be helpful in examining exposure, such approaches can be more challenging to interpret in patients with acute infection. Significant variability in the kinetics and magnitude of the serological response, especially early in infection, could contribute to false negative results. In addition, while both NAT and serological assays can suffer from inadequate analytical sensitivity, a broader range of factors can influence the sensitivity and specificity of serological tests, including a variety of platform design considerations such as the antigen coating density, dilution matrix considerations and the actual serum dilution employed. Challenges with false positives can also limit the value of serological tests. Cross-reactivity with

other strains of coronavirus[2], in addition to other pathogens altogether, can limit their accuracy. Consistent with this, during the 2003-04 SARS-CoV outbreak, various serological assays demonstrated cross-reactivity in assays that used whole virus in addition to the N protein. As a result, false positive serological results, which can be particularly challenging with IgM antibodies, can make it difficult to accurately interpret findings. Despite these considerations, the results of this manuscript suggest repeat serological testing may be informative in the acute setting, especially in patients who continue to follow a COVID-19-like disease course despite NAT negativity. The blood donation industry has used the approach of coupling the potential limitations and strengths of NAT and serological assays to detect pathogens in blood. A similar approach could increase the overall likelihood of identifying COVID-19 infected patients as shown by F Xiang et al[1].

While the results presented in this study provide some insight into how to possibly incorporate serological testing into the workup of acutely ill patients, especially if NAT test results are negative, how serology will be employed in screening asymptomatic individuals or patients who never underwent NAT testing during acute infection still remains unclear. For example, if someone is found to be serologically positive, should a follow up NAT test be performed to determine if the individual is shedding virus? Furthermore, the extent to which COVID-19 seroreactivity reflects actual immunity also represents an outstanding question. While established seroreactive corollaries with actual immunity exist, such as antibodies against hepatitis B surface antigen, these were developed over long periods of time. Furthermore, which populations would most benefit from COVID-19 serological testing remains unknown. Several populations may be good candidates:

1. Patients that have had COVID-19 compatible symptoms but have been NAT negative (as outlined in the present study[1]).
2. Populations in general to define the degree of community exposure which may help define the R null and mortality rate.
3. Frontline healthcare workers to likewise define exposure. Additional testing, such as NAT testing, may be warranted in serological positive individuals to determine potential risk for infecting others. It is important to reiterate that it is unknown the extent to which antibody positivity translates to actual immunity.
4. Convalescent patients that want to donate plasma for patients suffering from COVID-19 infection. Further testing to define optimal titer and overall neutralizing activity will be needed.

In short, the article of F Xiang et al. is a first step to better understand the antibody response against SARS-CoV-2 and in so doing provides important insight into the possible characteristics and use of serological tests in this ongoing pandemic. However, as other serological tests are developed, the analytical parameters of each of these tests will likely differ, making it difficult to fully extrapolate these findings to additional serological tests as they become available. Additional studies will therefore be needed to fully characterize serological platforms that are rapidly becoming available, both with respect to their analytical parameters and the role they may play in diagnosing and screening individuals for COVID-19. However, regardless of these considerations, this study provides important insight into the seroreactivity of patients with COVID-19 in general that will surely guide additional test development and use of serological testing moving forward.

Neither author has any potential conflicts to disclose.

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