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Using SARS-CoV-2 Antibody Testing in COVID-19 Research

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The novel Coronavirus Disease 2019 (COVID-19) pandemic is the first communicable disease in almost a century to surpass chronic and non-communicable diseases as the leading cause of death and morbidity worldwide.[1] Such unprecedented deep and widespread impact of an infectious disease in the modern era, which boasts of state-of-the-art medical accomplishments, is alarming and yet intriguing.

In the current issue, Ma et al. have investigated the association between physical activity and COVID-19 severity risks by identifying prior severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection as either self-reported or by presence of antibodies.[2] There is a fundamental vulnerability in utilizing antibodies to determine proof of prior infection, especially in secondary data analysis from large registries. We would like to provide critical yet constructive insight on SARS-CoV-2 antibody testing in COVID-19 research, that could augment characterization of prior COVID-19 infection or future scientific works.

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SARS-CoV-2 Antibody Testing

Since the early stages of the pandemic, the use of antibody testing has been lauded as well as criticized.[3, 4] A straightforward reason being that immunological response is a complex process and comprises of components which are regulated and or influenced by matrixed physiological and biochemical inputs from throughout the body. With SARS-CoV-2 being a novel virus, there are still many knowledge gaps 3-years into the pandemic. Reverse transcription polymerase chain reaction (RT-PCR) remains a gold standard for the diagnosis of current infection. However, there lacks a consensus in the scientific and medical community whether prior infections can be accurately identified either by leveraging current biomedical techniques or by development of innovative ones.

There are four structural proteins encoded by the SARS-CoV-2 genome, of which two are of high interest in determining antibody reactivity – membrane glycoprotein Spike (S) and nucleocapsid (N).[5] S-protein is the surface protein primarily involved in binding to host cells,

whereas N-protein is involved in penetrating the host's nucleus and promoting virus replication. Historically, S-protein specific immunoglobulin (Ig) (IgG, IgA, or IgM) antibody measurement has been the mainstay of assessing SARS-CoV-2 immunological response.[6] Enzyme-linked immunoassay (ELISA) testing of blood samples is an affordable and efficient mode of qualitative assessment of S- and N-specific antibodies, and therefore, a favorable screening tool for SARS-CoV-2 specific antibody status. Of these, anti-S IgG antibodies are mainly utilized in clinical and scientific applications owing to S-protein being abundant, larger, and easily detectable in serum samples. Additionally, S-protein is commonly targeted by neutralizing antibodies to detect infected cells,[7] which makes them further attractive for diagnosis and potential drug development.

Anti-S antibodies are produced by the lymphatic system as a response to the Spike protein, which is either found on the virus surface envelope or artificially supplied by vaccines (mRNA, vector, or protein subunit). More than 13 billion COVID-19 vaccine doses have been administered worldwide,[1] which confounds whether the anti-S antibodies found in peripheral blood are those acquired due to vaccination versus those in response to prior infection. On the other hand, anti-N antibodies (also produced by lymph nodes), are produced in response to SARS-CoV-2 genomic RNA-nucleocapsid complex formation inside the cell nucleus. This complex supports virion transcription, assembly, and release. Therefore, anti-N antibodies are formed only in response to viral invasion or replication and not secondary to vaccination. Thereby providing a small window of opportunity to distinguish the source of SARS-CoV-2 specific immune response between natural infection versus vaccination.

Limitations of SARS-CoV-2 Antibody Testing

The U.S. Centers for Disease Control and Prevention has provided succinct guidance on the utility and limitations of using COVID-19 antibody testing for diagnosis as well as outcomes.[8] Although few epidemiological studies have suggested the use of anti-S and anti-N IgG

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antibodies to distinguish between vaccination and prior infection, there are major pitfalls to this approach.

First, seroreversion (waning or loss of antibody detectability) occurs in all individuals and is not uniformly distributed over time. Post-infection, antibody levels significantly decrease by ~6-months and may even become undetectable in many cases. Longitudinal scientific data from multiple clinical trials and observational studies, which has been incorporated by the CDC and Food and Drug Administration (U.S. FDA), have determined that post-vaccination antibody levels wane significantly by ~8-10-months. These findings are the *principalis causa* for regular booster doses. Therefore, the mere absence of S- or N-antibodies is a non-reliable metric to determine “no prior infection/vaccination”. Second, not all individuals produce a sufficient antibody response to infection or vaccination (seroconversion). In fact, individuals with pre-existing chronic health conditions have a multifold higher likelihood of being seronegative (absence of Spike IgG) even after completing full vaccination regimen.[9] Third, seroconversion timeline is not uniform across all individuals. Although public health guidelines recommend a minimum 14-day incubation period after final vaccination dose or infection diagnosis for developing measurable antibody levels, there are multiple reports of individuals seroconverting well after that timeframe. Fourth, sero-reactivity will differ among different variants given the varying number of mutations on Spike protein (e.g., delta and omicron), which could potentially affect seroconversion lag-time. Moreover, we also need to consider whether IgM or IgA (S or N specific) can provide better information and whether the reliability of any of these markers is different during various phases of infection. Recent findings have shown that mucosal anti-S IgA could provide clinically relevant protection during early phases of SARS-CoV-2 infection, and this response is more robust and long-lasting than anti-S IgG.[10, 11]

Furthermore, seroconversion is a unit of measurable antibody levels (S or N) and not a measure of developing an appreciable antibody/immune response that can provide successful virus neutralization. There are ongoing contentious debates whether even neutralization titers

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are a strong correlate of actual protection (clinical outcomes). Given such matrixed and layered construct of immune response, relying solely on serological assessment of S- or N-IgG antibodies risks delivering incomplete and potentially misleading information to the public.

ELISA is a good primary approach to ascertain an adequate threshold of S- or N-antibodies (seropositive). It is affordable, quick, and requires minimum upheaval of laboratory setup and hence a perfect screening tool. But the information gained from serology, without knowing the neutralizing potential of these antibodies (primarily S-antibody), is incomplete. Measuring neutralizing titers (on pseudo virus or attenuated live virus) among those with seropositive status is expensive and cumbersome as it requires development and implementation of hefty biosafety level-3 (BSL-3) laboratory protocols, access to infectious material, and involves a considerable amount of time. Although seropositive status is positively correlated with microneutralization and plaque reduction neutralization assays, serology is unable to describe the full effect-size of the immune response, which may be important indices of COVID-19 research.[12] As mentioned above, the strength of neutralizing titers as actual correlate of protection remains to be established. Additionally, with the spurge of highly contagious variants and their sub-variants, virology experts are currently studying the feasibility and utility of serology and monoclonal antibody reagents to determine the degree of actual immunity (protection). Any such breakthrough will have to be detailed yet broad-based and obviously must stand the test-of-time

In conclusion, SARS-CoV-2 antibody measurements are powerful and informative tools with some glaring limitations. Clinical (RT-PCR) confirmation or self-reported disease are clearly necessary to augment the meaningful use of any SARS-CoV-2 antibody data. Secondary analysis of large registry data need to incorporate models with deeper appreciation of the complex yet important immune response. Only then can we move the needle towards accurate interpretation and dissemination of antibody results in COVID-19 research. Vaccination saves lives!

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