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MINIREVIEW

Immunity to SARS-CoV-2: What Do We Know and Should We Be Testing for It?

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ABSTRACT Preexisting immunity to Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) was nonexistent in humans, which coupled with high transmission rates of certain SARS-CoV-2 variants and limited vaccine uptake or availability, has collectively resulted in an ongoing global pandemic. The identification and establishment of one or multiple correlates of protection (CoP) against infectious pathogens is challenging, but beneficial from both the patient care and public health perspectives. Multiple studies have shown that neutralizing antibodies, whether generated following SARS-CoV-2 infection, vaccination, or a combination of both (*i.e.,* hybrid immunity), as well as adaptive cellular immune responses, serve as CoPs for COVID-19. However, the diverse number and type of serologic assays, alongside the lack of cross-assay standardization and emergence of new SARS-CoV-2 variants with immune evasive characteristics, have collectively posed challenges to determining a robust CoP 'threshold' and for the routine utilization of these assays to document 'immunity,' as is commonly done for other vaccine preventable diseases. Here, we discuss what CoPs are, review our current understanding of infection-induced, vaccine-elicited and hybrid immunity to COVID-19 and summarize the current and potential future utility of SARS-CoV-2 serologic testing.

KEYWORDS antibodies, COVID-19, correlate of protection, SARS-CoV-2, serology, vaccines

Since the early days of the COVID-19 pandemic, questions related to immunity against SARS-CoV-2 have persisted. From questions such as "Am I immune?" or "How long does immunity last?" to "What is 'better' – vaccine-or infection-induced immunity?" and "Why aren't we testing for immunity?," public and media attention has not wavered on this topic. In an effort to answer these questions and more, the scientific community has continued to investigate, at unprecedented speed, the complexities of the human immune response to the SARS-CoV-2. Now, 2 years after the first confirmed case of coronavirus disease-2019 (COVID-19), we have a much better understanding of SARS-CoV-2 infection-elicited, vaccine-elicited and 'hybrid' immunity, although numerous unknowns remain. For example, although functional neutralizing antibodies (nAb) can serve as a correlate of protection (CoP, defined below), a standardized nAb protective 'threshold' has not been (and may not be) determined (1, 2). Also, while we have a plethora of serologic assays with Food and Drug Administration (FDA) Emergency Use Authorization (EUA), these assays were developed to assess prior SARS-CoV-2 infection status, not immunity status. These assays currently have limited cross-assay correlation and lack standardization, despite the availability of a World Health Organization (WHO) international standard for anti-SARS-CoV-2 antibodies, and the majority of the assays detect binding antibodies (bAb), not specifically nAbs (3–5). Here, we provide a brief status update regarding our current understanding of immunity to SARS-CoV-2, focusing on humoral immune responses, CoPs, and the application of currently available SARS-CoV-2 serologic tests to determine antibody levels and immunity.

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CORRELATES OF PROTECTION

The precise definition and use of terms such as 'correlate' or 'surrogate' of protection have differed between experts and publications, complicating the already complex fields of immunology and vaccinology (6, 7). In an effort to standardize these concepts, Plotkin and Gilbert (2012) proposed that a correlate of protection (CoP) be defined as an immune marker that is statistically correlated with vaccine efficacy, and that CoPs can be either mechanistic or non-mechanistic in function (6). A mechanistic CoP is a marker that is 'mechanistically and causally responsible for protection,' whereas a non-mechanistic CoP is a predictor of protection, although it does not directly cause or lead to protection (i.e., a surrogate marker of protection). As an example of how mechanistic and non-mechanistic CoPs differ, consider vaccination against varicella-zoster virus (VZV). While both humoral and cellular immune responses have been correlated with vaccine efficacy, the latter has been shown to have both biologic functionality and a higher statistical correlation with protective efficacy compared to the humoral response (6, 8). As a result, cellular immune markers for VZV are mechanistic (functional) CoPs, whereas the humoral CoP for VZV, which is currently defined as an antibody titer $\geq$5 IU/mL, is considered a non-mechanistic correlate (9, 10). Despite the non-mechanistic nature of the VZV humoral CoP, given assay accessibility, standardization and ease of use, measurement of anti-VZV antibodies to determine protective immunity in a patient, is preferable to routinely measuring VZV cellular immune markers (e.g., interferon gamma release assays).

The identification of a CoP for vaccine preventable diseases (VPDs) is valuable from both the individual patient and public health perspectives. For individual patients, clinicians can assess immune status and determine whether vaccination or revaccination would be beneficial, particularly for those who may be at higher risk for disease (e.g., HBV vaccination of health care workers, rabies vaccination for veterinarians, etc.). At the public health level, having a CoP with a known protective threshold allows for more accurate assessment of population-level immunity (versus general seroprevalence studies). Additionally, defined CoPs allow for vaccine manufacturers to more readily determine vaccine consistency and efficacy, without the need for large clinical trials, and are beneficial in situations when assessment of a new vaccine may be unethical, due to the availability of an alternative, standard of care vaccine (9, 11).

The establishment of CoPs against infectious agents is challenging however. Although a detailed discussion of these challenges is beyond the scope of this article, they include the absence of a standardized method for how CoPs are determined. Many of the current VPD CoPs were identified using a variety of approaches over the past few decades, ranging from observational studies pre- and post-outbreaks (e.g., measles), to self-inoculation studies (e.g., tetanus), animal challenge studies (e.g., Yellow fever virus), passive immunization (e.g., Hepatitis A virus) and vaccine efficacy trials (e.g., VZV) (9, 12). Further complicating matters, is that the CoP(s) for a specific pathogen can vary depending on the desired clinical endpoint (i.e., limit infection versus disease severity versus death), the encountered pathogen challenge dose, the role of immune memory, individual patient demographics, including overall immunostatus, and the method used to detect the CoP. Vaccine-specific CoPs can also differ in the degree of protection they provide. CoP thresholds that are associated with nearly guaranteed protection against infection or disease (i.e., 'absolute' CoPs) have been identified for vaccines against tetanus ($\geq$0.1 IU/mL), diphtheria ($\geq$0.1 IU/mL), measles (200 mIU/mL) and rubella (15 IU/mL) (9). In contrast, certain VPD CoP thresholds are indicative of frequent, but imperfect protective immunity (i.e., 'relative' CoP). This is exemplified by the influenza vaccine, where a hemagglutination inhibition titer of $\geq$1:40 is protective in approximately 70% of patients, with increasing efficacy observed at higher titers (13).

IS THERE a COP FOR SARS-COV-2?

Although there is as of yet no agreed upon 'threshold' associated with protective immunity to SARS-CoV-2, a multitude of studies have now demonstrated that elevated anti-SARS-CoV-2 antibody levels, induced by either infection or vaccination, are correlated with decreased risk of subsequent symptomatic disease, and therefore antibodies are

considered a CoP for SARS-CoV-2 (1, 2, 14–17). Additionally, given the efficacy of monoclonal antibody therapy in patients, and the successful protection of human and nonhuman primates from SARS-CoV-2 following passive immunization with convalescent immunoglobulin, it can be argued that select anti-SARS-CoV-2 antibodies (i.e., nAbs), provide a mechanistic CoP (18–21). Antibodies can inactivate pathogens through a variety of means, including opsonization, complement activation and neutralization, the latter of which is particularly relevant for viral pathogens. With respect to SARS-CoV-2, a key nAb target is the receptor-binding domain (RBD) on subunit 1 of the spike (S) glycoprotein (S1); nAbs binding at this site effectively inhibit viral interaction with the angiotensin converting enzyme 2 (ACE2) receptor on host cells, ultimately limiting viral replication. A second important SARS-CoV-2 target for nAbs is the spike glycoprotein N-terminal domain (NTD), which when bound, is unable to undergo the necessary conformational changes for successful viral post-attachment steps, likewise resulting in limited viral replication (22).

Although the remainder of the manuscript will focus on the humoral CoP for SARS-CoV-2, given the interwoven nature of the immune system, the identification of one CoP does not exclude the possibility and even likelihood, that certain components of the cellular immune response may also serve as a CoP(s). While cellular immune mediators (i.e., CD4 and CD8 T-cells) do not directly inactivate viral pathogens (as is the case of nAbs), they do limit the consequences of infection by detecting and eliminating virally infected cells. Multiple studies have demonstrated that patients with mild or asymptomatic COVID-19 more frequently exhibit a rapid, initial expansion of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T-cells, which appear to be functionally superior to those generated in patients who progress to more severe disease (23, 24). Additionally, among patients with B-cell and/or other humoral immune deficiencies, those with a more robust $CD8^+$ T-cell response exhibited less severe COVID-19 outcomes (25). The importance of $CD8^+$ T-cells was also elegantly shown in rhesus macaques, where depletion of this cellular immune component led to lower protective efficacy against SARS-CoV-2, even in the presence of passively transfused antibodies (19). Perhaps most striking however, is the observation that protective efficacy following BNT162b2 vaccination is observed as early as day 10 after the first vaccine dose, at a time when SARS-CoV-2-specific T-cells are present at high quantities and when nAbs are not yet consistently detectable (26). Collectively, these findings support the likelihood that adaptive cellular responses also serve as CoPs. Unfortunately, given the higher assay complexity associated with the measurement of cellular immune markers (e.g., IFN-γ release assays, SARS-CoV-2-specific T-cell detection via flow cytometry, etc.), compared to antibody detection using routine serologic methods (enzyme-linked immunosorbent assays [ELISAs], chemiluminescent immunoassays [CIAs]), alongside the lack of a defined cellular CoP, cellular immune response assays for SARS-CoV-2 have not been widely adopted or implemented in clinical laboratories.

TESTING FOR ANTIBODIES TO SARS-COV-2

The literature is now replete with studies assessing antibody detection for SARS-CoV-2, with over 7,900 manuscripts published to date. There are currently over 85 serologic assays that have received FDA EUA. These vary in design, performance characteristics, the immunoglobulin class(es) detected and the SARS-CoV-2 antigen targeted (i.e., spike versus nucleocapsid) (27, 28). While we refer readers to previously published studies for further information on those topics, for the purposes of this review, it is important to recognize two key factors with respect to the available serologic assays. First, although an increasing number of SARS-CoV-2 serologic assays offer quantitative assessment of antibody levels, currently only one, the Ortho-Clinical Diagnostics VITROS Anti-SARS-CoV-2 IgG Quantitative chemiluminescent (CIA; Rochester, NY) is calibrated to the WHO SARS-CoV-2 antibody international standard (IS; NIBSC 20/136), and reports antibody levels as 'binding antibody units per mL' (BAU/mL). This WHO SARS-CoV-2 antibody standard, first released in December 2020, consists of freeze-dried, pooled convalescent plasma from 11 recovered patients, and was evaluated in 44 laboratories across 15 countries using over 125 different serologic methods (29). The WHO assigned potency of this international standard is 250 International Units (IU) per

ampule for assays detecting nAbs specifically, due to the clear biologic function of this immunoglobulin subset. For bAb assays however, the WHO Expert Committee on Biological Standardization indicated that quantitative values based on this international standard be reported as 'binding antibody units' (BAU) until further interlaboratory data on performance of the standard across these assays is acquired (30). There are numerous benefits of calibrating and harmonizing quantitative serologic assays to a single standard, including the ability to subsequently compare antibody values across assays and laboratories (30). Standardization of SARS-CoV-2 serologic assay outputs, which are currently quite variable, can then be applied toward the assessment of immunogenicity for current and future vaccines, and hopefully, the establishment of universal protective antibody thresholds, similar to what is available for other VPDs.

The second factor to be cognizant of regarding commercially available serologic assays is that the vast majority of them detect bAb *and* functional nAbs, without differentiation between the two. As of the writing of the manuscript, there are only two assays with FDA EUA specifically targeting nAb detection, including a blocking ELISA from GenScript (Piscataway, NJ) and a competitive inhibition ELISA from InBios (Seattle, WA), with both providing qualitative results. Notably, these are both surrogate methods for classic nAb detection assays (i.e., plaque reduction neutralization tests [PRNTs]). PRNTs however are notoriously challenging to perform, with long turnaround times, and due to the use of replicating native virus, biosafety level three (BSL-3) facilities are required for certain viruses, including for SARS-CoV-2. As a result, alternatives to PRNTs, have been developed, including recombinant pseudovirus neutralization assays, which utilize a less pathogenic viral backbone, such as vesicular stomatitis virus or a lentiviral vector, expressing the SARS-CoV-2 S protein (31–33). Although still technically challenging to perform, these assays can be completed at BSL-2 containment, and depending on the method and reporter system used, they allow for a quicker turnaround time to results. While these assays have been used extensively in the literature to evaluate cross-sectional or longitudinal nAb levels in vaccinated or previously infected patients, they lack standardization, making it difficult to draw overarching conclusions across methods and publications, including across vaccine efficacy clinical trials.

IMMUNITY FOLLOWING SARS-COV-2 INFECTION

Prior to the introduction of vaccines, the extent and duration of infection-elicited immunity to SARS-CoV-2 was the focus of many studies, which collectively showed that the response and kinetics of anti-SARS-CoV-2 antibodies can vary dramatically as a result of multiple factors, including disease severity, age, immunostatus and assay methodology (34–37). Despite these documented variables, the infrequency of reinfections, even with widespread ongoing transmission of SARS-CoV-2, suggested that primary infection provided some degree of protective immunity. This has now been supported by several large, prevaccine studies, which showed that seropositive individuals are at a significantly lower risk of contracting SARS-CoV-2 compared to seronegative individuals. Among these is a study by Lumley and colleagues who performed baseline anti-spike IgG antibody testing on over 12,000 health care workers (HCWs) in the United Kingdom and followed them by performing SARS-CoV-2 RT-PCR on nasal or oropharyngeal swabs bi-weekly and anti-spike/anti-NC serologic testing every 2 months for 31 weeks (16). Among the enrolled HCWs, 11,364 were seronegative and 1,265 were seropositive at baseline, of whom 223 and 2 individuals, respectively, had a positive SARS-CoV-2 RT-PCR result over the subsequent 7 months. The roughly 10-fold lower incidence of infection among seropositive individuals led to the conclusion that seropositivity is associated with a significantly decreased risk of infection over a 6-month period post-primary infection.

A second study followed over 3 million US patients with a documented anti-SARS-CoV-2 antibody result and monitored SARS-CoV-2 RT-PCR results in 30-day increments post-antibody testing (17). While the authors document a consistent 3–4% SARS-CoV-2 RT-PCR positivity rate among baseline seronegative individuals at all time points, at 90 days post-baseline serology, the RT-PCR positivity rate for seropositive individuals

was significantly lower at 0.3%. Similar to the Lumely et al. study, the sharp, 10-fold decline in SARS-CoV-2 RT-PCR positivity among baseline seropositive individuals indicates a protective effect of prior infection against both symptomatic disease and asymptomatic infection. Another study worthy of mention is by Hall and colleagues who also assessed the risk of symptomatic or asymptomatic reinfection among UK HCWs; however, they expanded follow-up to 12 months post-baseline serologic testing (38). The study enrolled 25,661 HCWs who were required to complete questionnaires and submit to SARS-CoV-2 RT-PCR and anti-spike IgG serologic testing every two to 4 weeks. Within the baseline seronegative cohort (N = 17,383), 1,704 acquired RT-PCR confirmed SARS-CoV-2 infection, compared to 155 RT-PCR confirmed reinfections among the 8,278 baseline seropositive HCWs over the study time frame. Similar to the aforementioned findings, this study concluded that a previous SARS-CoV-2 infection was associated with an 84% lower risk of reinfection over a 7-month period.

While these and other studies confirm the development of immunity against reinfection for a minimum of 6 months post-primary infection, there remain a few caveats. These include the limited understanding of cross-variant protection, which is increasingly important as we encounter new waves with emerging SARS-CoV-2 variants exhibiting significant amino acid substitutions in key neutralization motifs (e.g., B.1.1.529, omicron). Additionally, these studies were not designed to assess immunity relative to demographics or underlying comorbidities, they are based on population-level data which may not be generalizable to certain patient cohorts (e.g., immunosuppressed individuals), and are limited in the duration of enrollee follow-up, with minimal discussion on antibody or cellular immune response intensity or durability. These caveats, alongside the potentially severe and long-term consequences associated with SARS-CoV-2 infection, make the sole reliance on infection-induced immunity imprudent and ill-advised, especially given the availability of safe and effective SARS-CoV-2 vaccines.

IMMUNITY FOLLOWING SARS-COV-2 VACCINATION

Multiple different SARS-CoV-2 vaccine types have been developed, undergone clinical trials, and been granted Emergency Use Listing by the WHO or have been authorized or approved for use in the United States. These include mRNA vaccines (i.e., mRNA-1273, BNT162b2) and a non-replicating adenoviral vector vaccines (i.e., Ad26.COV.S, AZD1222), each expressing the SARS-CoV-2 wild type spike glycoprotein (39). Clinical trials for these vaccines, all performed prior to the delta and most recent omicron SARS-CoV-2 variant waves, demonstrated over 90% and 66% efficacy at preventing severe COVID-19 disease for the mRNA and Ad26.COV.S vaccines, respectively (14, 40, 41). While these trials monitored humoral immune responses among enrollees and documented nearly universal seroconversion, with high nAb geometric mean titers (GMTs) among participants, each trial used unique, non-harmonized serologic assays making cross-study comparison of bAb or nAb titers among vaccinees with and without breakthrough infections difficult.

In an effort to assess postvaccination antibody development and kinetics using commercially available SARS-CoV-2 serologic assays, Jeong et al. evaluated immune responses among 288 participants pre- and post-AZD1222 vaccination using six different semiquantitative or qualitative bAb and surrogate neutralizing assays (42). While they show seroconversion by all participants after the second dose and overall high qualitative correlation between the assays (kappa range 0.80–0.96), the fold difference in antibody levels ranged from 106-fold by the Elecsys anti-S total antibody electrochemiluminescent immunoassay (ECLIA; Roche Diagnostics) to 1.24-fold by the a nAb ELISA (SD Biosensor, South Korea), suggesting significant interassay measurement differences. More recently, Saker et al. assessed vaccine responses across multiple commercially available semiquantitative serologic assays, where output values were converted to BAU/mL relative to the WHO IS SARS-CoV-2 antibody standard, using manufacturer-provided conversion factors (5). Using 255 sera from 150 HCWs fully vaccinated with the BNT162b2 or AZD1222 vaccines, they show that the mean difference in resulting BAU/mL values among four quantitative assays differed anywhere from 10.6% to 60.9% between assays. The authors note that the largest discrepancy between quantitative titers, despite the application of WHO IS correction factors, occurred between assays

detecting different immunoglobulin classes against different SARS-CoV-2 antigenic regions. Collectively, while standardization to BAU/mL using correction factors appears to improve correlation between some assays, significant differences remain, underscoring the importance of using standardized serologic assay designs (i.e., same target analyte and antigen) and calibrating assays using primary or secondary standard material (rather than use of correction factors).

While the harmonization of quantitative serologic assays is now in progress, the value of these assays would grow exponentially should a defined correlate of protection 'threshold' be identified. Toward this goal, Bergwerk and colleagues monitored 11,453 HCWs, fully vaccinated with a two-dose BNT162b2 vaccine series, for breakthrough infection and matched each breakthrough event with four to five case-matched, uninfected controls (43). In this study, 22 HCWs developed breakthrough infection and were matched to 104 vaccinated, uninfected case controls. All 126 individuals had binding and neutralizing serologic test results available on samples collected within 1 week (peri-infection) of the first positive SARS-CoV-2 RT-PCR result, and 1 month after completion of the vaccine series. Comparison of antibody titers between breakthrough cases and controls showed lower nAb and bAb (detected by the Elecsys anti-S ECLIA) during the peri-infection phase among case patients, and although better differentiation between cases and controls was observed for nAb versus bAb, a specific protective titer could not be identified. More recently, using nAb and bAb quantitative serologic data (standardized to the WHO IS) from the mRNA-1273 coronavirus efficacy (COVE) clinical trial, Gilbert et al. show that anti-S IgG levels of 33 BAU/mL, 300 BAU/mL and 4000 BAU/mL at day 57 postvaccination correlated with 85%, 90% and 94% vaccine efficacy against breakthrough, symptomatic COVID-19 during the 4 months following receipt of the second mRNA dose (2). Importantly, the authors suggest that unlike for other VPDs, there does not appear to be a singular, absolute antibody level or threshold above which risk for COVID-19 disappears; rather the risk of disease is an inverse continuum, with incremental increases in antibody levels associated with decreased risk of disease. This is a seminal study in the search for a COVID-19 CoP, accentuating the significance of nAb and bAb, although a number of limitations remain, including the short postvaccination time frame evaluated and limited understanding regarding how these findings may change relative to new SARS-CoV-2 variants of concern.

Although SARS-CoV-2 vaccines induce strong humoral and cellular immune responses in otherwise healthy individuals, multiple publications have now shown that this is not the case among certain immunocompromised individuals. As an example, Addeo and colleagues assessed anti-spike antibody levels prevaccination and 22 days following completion of a two-dose mRNA vaccine series among 115 solid organ transplant (SOT) recipients and 25 patients with hematologic malignancies (HM) (44). While the authors documented an overall 94% seroconversion rate among all participants and no a significant difference in seroconversion rates based on vaccine manufacturer, patients with HM showed significantly lower seroconversion rates (77% versus 98%) and anti-spike antibody titers (832 U/mL versus 2,500 U/mL) compared to SOT recipients. They also showed that response to vaccination was significantly impacted by the type of therapy received, with individuals on cytotoxic regimens or mAb therapy having the lowest responses, with mean antibody levels of 611 U/mL and 152 U/mL, respectively, or not seroconverting at all (i.e., patients on anti-CD-20 mAb therapy). SARS-CoV-2 vaccine response can also vary relative to the specific type of hematologic malignancy, with minimal seroconversion occurring in patients with any of the non-Hodgkin lymphoma subtypes, whereas the majority of patients with acute or chronic myeloid leukemia show seroconversion by 14 days after administration of the second mRNA dose (range: 91% to 97.1%) (45).

Allogeneic stem transplantation in these patients has also been associated with weak serologic responses following a two-dose mRNA vaccine series (46). A third, booster dose however, has been associated with a significant improvement in antibody titers (47). Redjoul et al. compared anti-RBD antibody levels immediately prior to administration of the booster dose, which correlated with approximately 50 days after completion of a two dose mRNA vaccine series, and 26 days after boosting, in 42 patients who had received an allogeneic stem cell transplant (47). They documented a significant increase in median anti-RBD

levels post-boosting, from 737 AU/mL to 11,099 AU/mL. Importantly however, they note that only 48% of participants achieved antibody levels exceeding 4,160 AU/mL, which is considered a surrogate measure of vaccine protection according to the anti-spike assay manufacturer (Abbott Architect SARS-CoV-2 IgG Quant II, Abbott, Sligo, Ireland). Finally, it is important to note that despite limited antibody responses following vaccination of immunosuppressed patients, particularly those who are B-cell deficient, the majority of patients remain capable of developing strong cellular, T-cell mediated immune responses as measured by IFN-γ presence following stimulation with different spike antigen epitopes (48–50).

HYBRID IMMUNITY AGAINST SARS-COV-2

Hybrid immunity is defined as occurring in individuals who have been infected with and vaccinated against SARS-CoV-2. Multiple studies to-date have documented significantly higher postvaccination antibody responses in these individuals compared to SARS-CoV-2 naive cohorts, irrespective of vaccine manufacturer. Among the first of these studies was one by Krammer and colleagues who show that following a single mRNA vaccine dose, post-vaccine antibody titers in COVID-19 recovered individuals was 10 to 45 times higher than in SARS-CoV-2 naive individuals (51). Notably, while administration of the second mRNA vaccine dose led to a 3-fold increase in antibody levels among infection-naive patients, no increase was noted among previously infected individuals, who still showed a 6-fold higher antibody level. Using a commercially available, semiquantitative anti-S IgG CIA (Abbott Laboratories Inc., Lake Forest, IL), Ebinger et al. showed that COVID-19 recovered patients who received a single vaccine dose had statistically identical anti-S IgG levels compared to infection-naive patients following receipt of two mRNA vaccine doses (52). Both cohorts had bAb levels above 4,160 AU/mL by the Abbott CIA, which is a threshold identified by the manufacturer to represent a 95% probability of having high nAb titers and thus be predictive of immunity. Additionally, others have shown that vaccination of previously infected individuals leads to significant higher cross-variant neutralization (by 1000-fold) compared to fully immunized, infection-naive individuals (53). Based on these and other similar findings, the argument was made that while all individuals should be vaccinated, regardless of prior infection status, given the similar humoral immune responses, perhaps a complete vaccine series may be foregone in individuals with prior SARS-CoV-2 infection. Importantly however, as immune evasive variants such as omicron emerge, reliance on past infection and incomplete vaccination may not be sufficient to protect against hospitalization, leading the CDC and others to endorse complete vaccination and boosting for all elligible individuals (54).

The question however remains, whether infection-induced, vaccine-elicited or hybrid immunity, how long does protection from disease or infection last? Although individuals with hybrid immunity have been considered 'super-immune,' recent data from Israel indicate that regardless of how immunity is generated, it tends to wane. In a still preprint study, Goldberg and colleagues show that among previously infected individuals who received one BNT162b2 vaccine dose (hybrid immunity), SARS-CoV-2 infection cases increase from 10.5/100,000 risk days within 2 months of vaccination to 30.2/100,000 risk days more than 6 months after vaccination (55). In comparison, among infection-naive, two-dose BNT162b2 vaccinated individuals, SARS-CoV-2 cases increased from 21.1 to 88.9 per 100,000 risk days from the first 2 months postvaccination to over 6 months later, respectively. These results continue to reinforce that hybrid immunity, despite also waning over time, yields a lower infection risk compared to vaccination alone, and supports the recent push for vaccinees to receive a booster dose if at least 6 months post-completion of a primary vaccine series. Given that these data were largely acquired during the delta SARS-CoV-2 wave, the applicability of these findings to the new variant of concern, omicron, has been questioned. Although studies are still ongoing, preprint data indicate that the neutralization efficacy of sera against omicron collected from solely convalescent or fully (two dose) vaccinated individuals is dramatically lower (32- to 57-fold) or nonexistent for some vaccines (e.g., Sputnik, AD26.CoV2.S) compared to neutralization of the original SARS-CoV-2 strain (56, 57). Notably, individuals with either hybrid immunity due to prior, non-omicron related infections, or those who have received three doses of mRNA-1273,

TABLE 1 Optimization needs and clinical role for SARS-CoV-2 serologic testing[a]

Optimization needs:
Standardize quantitative immunoassays to international standard
Identify clinically relevant antibody quantitative value(s)
Standardize immunoassay design to targeted immunoglobulin class and SARS-CoV-2 antigen
Current assays detect IgM, IgG, or total antibodies against NC, RBD, S1, trimeric S, etc.
Confirm immunoassay sensitivity to emerging SARS-CoV-2 VOCs exhibiting significant mutations in the viral antigen used by the assay
Current and possible future clinical role(s):
Support diagnosis of COVID-19 in select patients (Current; [60])
Identification of past infection to support the diagnosis of certain COVID-19 sequelae (Current; [60])
Use anti-NC immunoassays (except among individuals vaccinated with inactivated SARS-CoV-2)
Qualify high-titer COVID-19 convalescent plasma (Current/Future; [62])
Bridge therapy for immune evasive SARS-CoV-2 VOCs resistant to available monoclonal antibody therapies
Use of anti-S immunoassays with established threshold for 'high-titer' CCP
Identify sufficient, postvaccination humoral immune response (Future)
Beneficial in select, immunocompromised patient populations that may benefit from additional booster(s)

[a]NC, nucleocapsid; RBD, receptor binding domain; S1, spike glycoprotein subunit 1; S, spike glycoprotein; VOC, variant of concern.

retain high neutralization efficacy of omicron, with only slight drops in nAb titers relative to the wild-type strain (4.2- to 5-fold decrease) (56–58).

TO TEST OR NOT TO TEST FOR ANTIBODIES AND IMMUNITY TO SARS-COV-2? That REMAINS THE PERSISTENT QUESTION

Our understanding of the many facets of immunity to SARS-CoV-2 infection and COVID-19 likely rivals our understanding of immunity against any other infectious disease studied to date, and we continue to learn more with every emerging variant and disease state. Despite these advances, the role of routine, blanket testing for humoral immune responses to SARS-CoV-2 remains of limited clinical value (at this time). There are of course clear indications for the use of humoral and cellular immune assays for vaccine or other SARS-CoV-2-related clinical trials and research studies. It is imperative however, that trials which monitor humoral immune responses utilize quantitative serologic assays calibrated to an international SARS-CoV-2 antibody standard, such as the one released by the WHO (NIBSC 20/136) in late 2020 (30). At a minimum, this will allow for simpler cross-study comparisons and potentially, for faster implementation of new guidelines or policies should correlates of protection be further defined.

The role of serologic testing in the clinical setting however, has not changed dramatically since the addition of these assays to our testing arsenal at the start of the pandemic (59). The Centers for Disease Control and Prevention (CDC) SARS-CoV-2 antibody testing guidelines continue to indicate their role for detecting recent SARS-CoV-2 infections in patients with more than 7 days of symptoms who test negative by a SARS-CoV-2 diagnostic assay, and for helping to diagnose long-term COVID-19-associated sequelae. Regarding the latter scenario, anti-NC-based SARS-CoV-2 immunoassays would be preferable in this use-case, to differentiate between infection and vaccine-induced immune responses (Table 1) (60). Although COVID-19 convalescent plasma (CCP) is no longer frequently administered due to the availability of monoclonal antibody therapy, the immune evasive properties of omicron have rendered all but a few of these therapeutic products ineffective (56, 61). As a result, there is renewed interest in use of serologic assays to identify high-titer, recently collected CCP, which presumably has greater neutralization activity against circulating variants, to treat patients during the current omicron wave (62). Use of serologic assays, as a means to determine an individual's response to SARS-CoV-2 vaccination and immunity however, continues to be discouraged by the CDC, FDA and other national and international organizations, although public interest for such testing and test availability remain high. The application of serologic assays to measure 'immunity' to SARS-CoV-2 in the future

would be possible however, should these methods undergo a certain level of optimization and standardization as discussed above (i.e., use of quantitative, anti-spike-based assays detecting IgG-class antibodies reported as U/mL) and should definitive CoP(s) be defined.

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