Journal of Infectious Diseases & Therapy

Heffernan, et al. J Infect Dis Ther 2021, 9:4

Review Article Open Access

The Role of SARS-Cov-2 Antibody Testing; Current and Future Applications

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Received date: June 01, 2021; **Accepted date:** June 15, 2021; **Published date:** June 22, 2021

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Abstract

SARS-CoV-2 which causes coronavirus disease (COVID-19) is still challenging health care systems and governments all over the world. Although molecular testing remains the most reliable laboratory method available for establishing active infection, serological tests can identify past infection and measure immune response in vaccinated individuals. Serological tests have a number of useful applications in the management and control of the COVID-19 pandemic including as indicators of past infection, an adjunct to molecular testing in certain clinical situations, the diagnosis of late presentation COVID-19, seroprevalence studies and in assessing the efficacy of vaccines in development and the follow-up and monitoring of vaccinated individuals. Initial SARS-CoV-2 antibody assays which were qualitative and had different antigens as their target proteins have been revised to include the Spike (S) protein or the Receptor Binding Domain (RBD) protein as their target antigens and have the potential to quantify the antibody response. These assay revisions will facilitate the quantification of specific antibody titre which will in turn enable the monitoring of antibody response in individuals over time and the response to different available vaccines. Serological assays designed to assess antibody response to vaccination should include a good correlation of antibody results with neutralizing activity and evidence suggests that S protein based immunoassays correlate better with neutralizing activity than Nucleocapsid (N) protein based assays. Studies comparing both the N and S protein based assays have shown an additional utility of serological assays in differentiating between a SARS-CoV-2 infected antibody response and a vaccine induced immune response. Additionally, quantitative antibody assays could play a role in a more targeted distribution of vaccines by assessing antibody levels after one dose of vaccine which will allow for wider vaccine coverage. Standardisation of SARS-CoV-2 antibody assays is essential and the recent availability of an International Standard (IS) and International Reference Panel for anti-SARS-CoV-2 immunoglobulins will facilitate the future development and evaluation of serological assays. This will in turn help define protective levels of antibody and aid in the assessment of the efficacy and durability of antibody responses to the different vaccines available and in development.

Keywords: SARS-CoV-2 antibody; Serological tests; Quantitative assay; Neutralizing antibody; International standard

Introduction

Since its discovery in December 2019, coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still challenging health care systems and governments all over the world. Since being declared a pandemic by the WHO in March 2020, COVID-19 has infected over 179 million people worldwide resulting in approximately 3.89 million deaths [1]. Attempts to contain the virus have included mass quarantine, isolation of infected individuals, enhanced hygiene strategies and serial lockdowns which have all had significant economic implications globally. Molecular testing for the diagnosis of acute SARS-CoV-2 infection remains the most reliable laboratory method available for establishing active infection and has been used as the gold standard for the primary diagnosis of acute SAR-CoV-2 with specific detection of viral RNA by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). There are currently two other SARS-CoV-2 testing methods available that have been utilized in the management of the ongoing COVID-19 pandemic; antigen tests that measure SARS-CoV-2 proteins and can quickly identify infectious individuals with transmissible virus and serological tests to identify possible past infection and measure immune response in vaccinated individuals. Serological tests, while not suited for the early diagnosis of SARS-CoV-2 infection, have a number of useful applications in the management and control of the COVID-19 pandemic and represent a cost-effective and easily implemented way of identifying individuals who have previously been exposed to the SARS-CoV-2 virus. The International Federation of Clinical Chemistry (IFCC) has previously recommended serological tests as valuable indicators of prior infection in both non-hospitalized patients (symptomatic and asymptomatic) and when assessing community exposure to the virus *via* seroprevalence studies.

They also emphasized that antibodies against SARS-CoV-2 serve as an addition to molecular testing in patients who have symptoms suggestive of COVID-19 and are RT-PCR negative and patients with a persistent positive RT-PCR in the absence of infectious virus [2]. Serology testing has also been identified as a useful tool in the diagnosis of COVID-19 in so-called 'late' presentation patients who present two weeks or more after symptom onset and in patients with prolonged symptoms [3]. A recent paper by Plebani, which reviewed studies assessing the serorevalence of SARS-CoV-2 infections in

different regions, suggests that there is a significant risk of underdiagnosing SARS-Cov-2 infections when using RT-PCR alone and highlights the value of serological assays in understanding the spread of SARS-CoV-2 infection in specific populations [3]. The CDC guidelines on SARS-CoV-2 antibody testing suggest that current evidence is still limited and serological tests can vary in their performance characteristics although they do suggest that antibody testing may be useful to support the diagnosis of COVID-19 illness or complications of COVID-19 in certain situations [4]. Plebani, when reviewing a number of studies on seroprevalence of COVID-19 in different regions worldwide suggested that further studies are needed to establish the true value of SARS-CoV-2 antibody testing in clinical practice [3].

Literature Review

Currently with the advancement of the rollout of numerous different vaccines, antibody assays will play a further fundamental role both in the follow up of vaccinated individuals by monitoring their immune response and in the development of further vaccines by assessing the efficacy of vaccines in clinical trials. Vaccine development has progressed at speed and as more vaccines are approved, there is a growing need for highly specific and sensitive serological assays for supporting COVID-19 diagnosis, for seroprevalence studies and to estimate the quality and quantity of the immune response to the different vaccines [5].

Serological immunoassays can be categorized by the antibody isotype and the type of antigen identified. Initial SARS-CoV-2 antibody assays were designed to identify individuals with previous exposure to the SARS-CoV-2 virus and distinguish them from individuals who had no prior exposure to the virus. These original commercially available serological assays were qualitative assays and used various antigens as the target protein including the nucleocapsid protein (N), the spike protein (S) and the Receptor Binding Domain of the spike protein (RBD). While these assays have been very valuable in seroprevalence studies and assessing previous exposure to the virus, additional applications of qualitative assays are limited. Our study evaluated a range of these preliminary SARS-CoV-2 antibody assays and concluded that the Fortress SARS-CoV-2 Total antibody ELISA assay demonstrated the highest sensitivity and specificity followed closely by the Roche ECLIA assay which had the advantage of being a fully automated assay [6]. Subsequent studies on these SARS-CoV-2 antibody assays have confirmed these results with the Fortress/Wantai total antibody ELISA demonstrating the highest sensitivity and specificity in detecting SARS-CoV-2 antibodies post-exposure to the virus following an evaluation of nine commercial serological assays in a cohort of COVID-19 patients with mild symptoms [7]. In addition, a more recently published study on the performance characteristics of four fully automated SARS-CoV-2 CLIA antibody assays had similar results to our study with the Roche Elecsys Anti-SARS-CoV-2 N assay demonstrating the highest sensitivity and specificity out of the four high-throughput assays evaluated (Roche Elecsys Anti-SARS-CoV-2, Abbott Anti-SARS-CoV-2 IgG, Siemens SARS-CoV-2 Total and Siemens SARS-CoV-2 IgG). This study was the first published independent evaluation of the Siemens COV2G assay which demonstrated an un-expectantly low sensitivity and inferior performance to the other assays evaluated [5].

The more recently developed SARS-CoV-2 serological assays are designed not only to identify individuals with previous exposure to the SARS-CoV-2 virus but also to monitor and measure the humoral immune response to COVID-19 vaccinations in individuals. These assays have been revised to include the correct target antigen (S-protein or RBD protein) and they also have the potential of quantification of the results. Quantification of the antibody response can help to establish specific antibody titre which can aid in monitoring the antibody response in individuals over time, monitoring antibody responses to vaccines and in comparing the differences in the different vaccines available and between different risk groups [8]. The limited published evidence on the performance characteristics of these newer commercially available quantitative assay evaluations suggests comparable if not superior results in terms of sensitivity and specificity of the assays. In a recently published review of five automated SARS-CoV-2 serology immunoassays the Roche Elecsys Anti-SARS-CoV-2 S assay demonstrated a higher clinical sensitivity than its predecessor the Elecsys Anti-SARS-CoV-2 N assay but a lower specificity [9]. This study proposed that in high prevalence settings, the Roche S assay could be considered without a secondary confirmatory test and in lower pre-test probability settings the Roche N and Roche S assays could be combined for optimal diagnostic performance [9]. A clinical evaluation of the Abbott Alinity SARS-CoV-2 quantitative IgG and IgM assays in a range of patient cohorts including vaccinated individuals demonstrated a specificity of 100% and a sensitivity of 100% in both the IgG and IgM assays individually [10]. An evaluation on the recently released Siemens SARS-CoV-2 IgG (sCOVG) by the same research team that carried out the previously mentioned study on the performance characteristics of four fully automated SARS-Cov-2 CLIA antibody assays demonstrated improved sensitivity compared to the previous Siemens COV2G assay with no differences in specificity observed [11].

A further important application of serological tests is to understand the different antibody responses mounted after both natural infection and vaccination. Of particular significance is the evaluation of the neutralizing antibody response and the primary goal of vaccination is to induce neutralizing antibodies. Neutralising antibodies bind to the virus and prevent infection and therefore are of particular importance in determining whether antibodies are effective in providing protective immunity. The relationship between SARS-CoV-2 antibodies and efficient neutralizing activity remains unclear and detection of SARS-CoV-2 antibodies is not an indication as to whether the antibodies are functional for neutralizing the virus. Antibodies directed against N antigens are produced early and strongly in infected individuals however they are unlikely to be functionally relevant in providing protective immunity. Antibodies that react with the S protein however are likely to have the function of neutralizing antibodies and evidence suggests that S protein based immunoassays correlate better with neutralizing activity than N protein based assays [12] and many of the vaccines developed focus on eliciting an immune response to the RBD of the spike protein.

A fundamental prerequisite for serological assays designed to measure the humoral response to vaccination include a good correlation of antibody results with the presence of neutralizing antibodies. Current literature suggests that levels of SARS-CoV-2 antibodies detected by immunoassays should be closely correlated with neutralizing antibodies and only if correlation is demonstrated should they be used as a way of measuring neutralizing ability [13]. The previously mentioned study on the evaluation of the recently released Siemens SARS-CoV-2 IgG (sCOVG) assay which detects antibodies against the S1-RDB antigen also demonstrated good correlation of the sCOVG result with a SARS-CoV-2 neutralization assay. In addition quantitative results for S-RBD IgG levels

determined with this assay correlated with SARS-CoV-2 neutralization titres and the severity of COVID-19 [11]. A recently published study comparing the performance of commercially available serological assays to detect antibodies to SARS-CoV-2 and identify individuals with high neutralizing titres demonstrated that the Roche Elecys Anti-SARS-CoV-2 N and Abbott Anti-SARS-CoV-2 N assays had the highest diagnostic accuracy for the detection of antibodies and had better specificities than the ELISA assays evaluated however the Roche assay ratios weakly correlated with neutralizing antibody titre and poorly identified convalescent individuals with high neutralizing antibody titres [14]. The Euroimmune ELISA Anti-SARS-CoV-2 IgG S protein assay demonstrated the best correlation with neutralizing antibody titres and performed better in differentiating high neutralizing antibody titres than the Roche and Abbott assays. The Euroimmune assay was the only assay in this study which used the spike protein as the target antigen for detection of antibodies [14] which further supports the evidence that S protein based immunoassays correlate better with neutralizing activity than N protein based assays and that these N based assays may have limited applications in measuring neutralizing ability and therefore limited use for measuring immune response in individuals post vaccination.

Studies involving comparisons of assays using the N protein as a target antigen and the S protein as the target antigen have identified an additional utility of serological assays in evaluation and distinguishing serological response to infection and vaccination. The study comparing the Roche Anti-SARS-CoV-2 N and the Roche Anti-SARS-CoV-2 S assays suggested that using a testing strategy which employs a combination of N and S target proteins can differentiate antibodies induced by SARS-CoV-2 infection (which would induce anti-N and anti-S antibodies) and vaccine-induced antibodies (which would induce only anti-S antibodies) [9]. Similarly when comparing the Abbott N antibody assay to the newer Abbott S specific quantitative assay were able to demonstrate a clear differentiation between a SARS-CoV-2 infected antibody response and a vaccine induced immune response [10]. Current CDC guidelines state that antibody testing is not currently recommended to assess for immunity to COVID-19 following vaccination or to assess the need for vaccination in an unvaccinated persons as post-vaccination serological results will be negative in persons without history of previous natural infection if the test does not detect antibodies induced by the vaccine [4]. Employing a testing strategy which uses assays targeting both the N and S proteins can distinguish a specific vaccine induced immune response and therefore establish whether an individual has had an antibody response to the vaccine.

Serological assays have various applications not only in the management of the COVID-19 pandemic but also in the long term follow up of the humoral antibody response as the durability of the antibody response to the SARS-CoV-2 virus as well as the extent and duration of immunity against reinfection are still under investigation [15]. Antibody assays will also have an increased role in the evaluation of the efficacy and durability of antibody response to the various different vaccines currently available and in development. Furthermore, employing a testing strategy which combines both N and S target proteins can differentiate between an immune response to SARS-CoV-2 infection and a vaccine-induced immune response [9,10]. Preliminary results on vaccine induced response suggests that one vaccine dose would suffice for individuals who have previously had COVID-19 infection [16,17]. Quantitative antibody assays could therefore potentially have a supplementary role in a more targeted distribution of vaccines by assessing antibody levels after one dose of vaccine thus prioritizing the administration of booster jabs for individuals with no previous infection which will allow for wider vaccine coverage. Furthermore the evaluation of antibody response after one vaccine dose could potentially avert adverse reactions as evidence suggests that individuals with pre-existing immunity had a higher incidence of side effects following one vaccine dose than individuals who had no pre-existing immunity [17].

Discussion

Standardization of available quantitative serological assays is vital and for assays to accurately quantify the immune response to the SARS-CoV-2 virus and to vaccination, universal reference antibody standards are essential. The International Standard (IS) and International Reference Panel for anti-SARS-CoV-2 immunoglobulins have recently been adopted by the WHO Expert Committee on Biological Standardization [18]. Availability of an IS will allow for the accurate calibration and standardization of SARS-CoV-2 serological assays and the reference panel will facilitate the future development and evaluation of serological assays. Standardization of serological assays will aid assessment of vaccine efficacy, help define protective levels of antibody and support the comparison of data collected from seroprevalence studies [19].

Conclusion

We conclude that the recent availability of both the international stand ard and reference panel for anti-SARS-CoV-2 immunoglobulins will allow for the much needed standardisation and accurate calibration of available SARS-CoV-2 serological assays which in turn will facilitate their further development and expand the future applications of these assays.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and publication of this article.

Contributor Ship

EH wrote the main article. All authors contributed and approved the final version of the article.

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