Correlation of SARS-CoV-2 neutralizing antibodies to an automated chemiluminescent serological immunoassay

Running head: SARS-CoV-2 neutralizing antibodies

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Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; NAbs, neutralizing antibodies; PRNT, plaque reduction neutralization test; EUA, emergency use authorization

Abstract

Introduction

Neutralizing antibodies (NAbs) are capable of binding to a virus to render incapable of infection. The ability of commercially available SARS-CoV-2 serological tests to detect NAbs has not been widely reported. We sought to correlate the antibodies detected by an automated chemiluminescent immunoassay with NAbs.

Methods

Residual serum samples from 35 patients that had a positive antibody test using the LIAISON® SARS-CoV-2 S1/S2 IgG chemiluminescent immunoassay and two antibody-negative control sera were tested for NAbs using a plaque reduction neutralization test (PRNT).

Results

NAbs were detected in 66% (23/35) of the antibody-positive samples. The immunoassay signal value ranged from 21.7 to 131.3 AU/mL (median, 90.5) with significant correlation between it and the PRNT ($r=0.61$, $p=0.002$). In the samples without NAbs, the immunoassay signal ranged from 16.3 to 66.2 AU/mL (median, 27.2). An immunoassay signal cutoff of >41 AU/mL was 91% sensitive and 92% specific for the detection of NAbs.

Discussion

It is important that correlates of immunity to SARS-CoV-2 be identified and NAbs are considered to be central indicators of such. PRNT is the gold-standard test for identifying NAbs but it cannot be used for large-scale testing of populations. It is

necessary to establish relationships between it and widely used commercial serological assays for SARS-CoV-2.

Impact Statement

Establishing relationships between the gold-standard plaque reduction neutralization tests and commercially available SARS-CoV-2 serological assays is necessary to help guide the clinical use of serological tests. This study helps to establish that relationship between one commercially available antibody test and results of a plaque reduction neutralization test.

Introduction

It is important that correlates of immunity to SARS-CoV-2 be identified and NAbs are considered to be central indicators of such. PRNT is the gold-standard test for identifying NAbs but it cannot be used for large-scale testing of populations. It is necessary to establish relationships between it and widely used commercial serological assays for SARS-CoV-2.

Shortly after the emergence of the novel coronavirus, SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19), many serological assays to assess the humoral immune response were developed. In the last few months, there have been many reports focused on the analytical and/or clinical performance of these assays (1,2).

The clinical usefulness of these antibody tests is limited to specific indications. These include use as a potential indicator of infection following a negative SARS-CoV-2 nucleic acid amplification test result in patients with symptoms of COVID-19 who present later in their illness, and the identification of individuals who have been previously infected with SARS-CoV-2 and who may be considered as convalescent plasma donors (3).

Currently, there is insufficient evidence that the presence of antibodies to SARS-CoV-2 confers immunity to subsequent reinfection. Until such correlates to immunity are well-understood, serological test results should not be used to make decisions regarding

decreased social distancing, return to work policies, or a decreased need for personal protective equipment.

Neutralizing antibodies (NAbs) are a subset of the humoral response to a viral infection that are capable of binding to a virus and rendering it incapable of infection. Infection with SARS-CoV-2 has been shown to elicit NAbs with specificity to the receptor binding domain of the viral spike (S) protein (4). The ability of commercially available SARS-CoV-2 serological tests to detect NAbs has not been widely reported. We sought to correlate the antibodies detected by the LIAISON® SARS-CoV-2 S1/S2 IgG (DiaSorin Inc., Stillwater, MN) chemiluminescent immunoassay with NAbs as determined by a plaque reduction neutralization test (PRNT).

Methods

Thirty-five residual, de-identified serum samples that had a positive antibody test and two antibody-negative control sera were included in this study. Use of the samples were approved by the Advarra institutional review board.

The chemiluminescent immunoassay qualitatively detects IgG antibodies directed against the S1 and/or S2 subunits of the viral S protein using a signal cutoff value of 15 AU/mL to differentiate positive (≥15 AU/mL) and negative (<15 AU/mL) results. The PRNT determines the titer of NAbs required to prevent infection of susceptible cells as determined by a reduction in plaque-forming units. In a biosafety level 3 facility, Vero-E6 cells were seeded in 12-well plates and incubated at 37ºC for 12-24 hours until at least

90% confluent. SARS-CoV-2 (isolate USA-WA1/2020), was diluted to 50-100 PFU/200 μL in viral growth medium (VGM, minimal essential medium with 2.5% heat inactivated fetal calf serum). Serum samples were complement-inactivated by heating at 56ºC for 30 minutes and then diluted in VGM beginning at 1:80 with serial 2-fold dilutions and mixed with equal volumes of diluted virus and incubated at 37ºC for 1-1.5 hours. 400 μL of serum-virus mixtures and virus controls were added to Vero-E6 cells and incubated for 2 hours at 37ºC. After incubation, virus was aspirated and cells were washed once with PBS. Cells were overlaid with 1 mL virus overlay medium (equal volumes of 2% agarose and 2x minimal essential medium concentrate supplemented with 5% fetal calf serum and 2x penicillin/streptomycin) and incubated at 37ºC for 2 days. Cells were fixed at 4ºC overnight with 4% formaldehyde. Fixative was aspirated and the viral overlay was removed. Cells were stained with 0.5% crystal violet for 1-2 minutes, washed, and dried. The endpoint titer was the serum dilution that reduced plaque formation by 80% compared to negative control sera (PRNT80). A titer ≥1:80 was considered positive for NAbs.

Spearman correlation was used to determine the relationship between the immunoassay signal and the PRNT titer of samples with detectable neutralizing antibodies. ROC curve analysis was determined using the LIAISON signal value from samples with and without NAbs. Data analyses were performed using Prism 8 (GraphPad Software, San Diego, CA).

Results

NAbs were detected in 66% (23/35) of the antibody-positive samples. The immunoassay signal value ranged from 21.7 to 131.3 AU/mL (median, 90.5) with significant correlation between it and the PRNT80 (r=0.61, p=0.002)(Figure 1). In the samples without NAbs, the immunoassay signal ranged from 16.3 to 66.2 AU/mL (median, 27.2). Receiver operator characteristic curve analysis produced an area under the curve of 0.924 (Figure 2). An immunoassay signal cutoff of >41 AU/mL was 91% sensitive and 92% specific for the detection of NAbs.

Discussion

Correlates of immunity to SARS-CoV-2 are critical to identify and the presence of NAbs is considered to be an important indicator of protective immunity for many viral infections, including SARS-CoV and MERS-CoV (5), two other highly pathogenic coronavirus. While it is not yet clear if NAbs are the predominate mechanism that confers immunity to SARS-CoV-2, they played an important role in the neutralization of SARS-CoV (6). While PRNT is considered the gold standard test for identifying NAbs, they cannot be performed for large-scale testing of populations. Thus, it is important to establish relationships between widely used commercial serological assays for SARS-CoV-2 and PRNT.

The immunoassay used in this study is specific for IgG. IgG, and potentially IgA, are likely the most important antibody classes in the neutralization of SARS-CoV-2 due to their long-lived responses and higher antigen affinity. However, it is possible that IgM antibodies, especially in acute patients, could contribute to neutralization. Indeed, in

Ebola virus vaccination in humans, IgM antibody has been shown to contribute significantly to neutralization responses (7). We and others have shown that IgM titers appear to be relatively transient in acute COVID-19 patients compared to IgG but are variable in different patient populations (8,9). There are conflicting data regarding whether IgM titers correlate with neutralizing titers in COVID-19 patients (10,11). Therefore, additional studies are needed to clarify the contribution of IgM to neutralizing of SARS-CoV-2 in COVID-19 patients.

On August 23, 2020, the US Food and Drug Administration issued an emergency use authorization (EUA) for the use of convalescent plasma for the treatment of patients hospitalized with COVID-19 (12). The authorization specifies that donated plasma must be tested for SARS-CoV-2 antibodies using the VITROS® Anti-SARS-CoV-2 IgG test (Ortho Clinical Diagnostics, Inc., Raritan, NJ). The reason for the requirement of that specific serological assay and no other is not given in the EUA. One study has reported a high degree of correlation (r=0.75) between the VITROS® Anti-SARS-CoV-2 Total assay and NAbs using 370 convalescent plasma samples, but that same study also demonstrated similarly high correlation (r=0.72) with the Architect SARS-CoV-2 IgG immunoassay (Abbott Laboratories, Abbott Park, IL) (12). It is anticipated that as more data correlating SARS-CoV-2 serological tests to NAbs, these assays would also be permitted to identify convalescent plasma donors.

Although the number of samples included in this study is somewhat limited, we demonstrate that the signal result of the LIAISON immunoassay shows significant

correlation to PRNT80 suggesting that it, too, is capable of detecting NAbs. It is possible, of course, that the antibody assay and PRNT are not detecting the same antibodies, and that the relationship between the two is coincidental. However, the significant correlation between the two tests argues against that hypothesis. Converting the sensitivity and specificity at a signal cutoff of >41 AU/mL into likelihood ratios yielded positive and negative likelihood ratios of 11 and 0.09, respectively, values that effect large changes on post-test probabilities.

This work helps to establish the needed relationships between high-throughput, automated SARS-CoV-2 serological tests and PRNT. These relationships are valuable assets in efforts to identify correlates of immunity to the novel coronavirus and could potentially assist in the identification of convalescent plasma for therapeutic use.

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Figure 1. Scatter plot of the serological immunoassay versus PRNT. Note the ordinate axis is a log scale. The dashed vertical line is the signal cutoff value of 15 AU/mL that differentiated positive and negative results. The dashed horizontal line is the PRNT titer of 1:80 that was used to identify samples positive for NAbs.

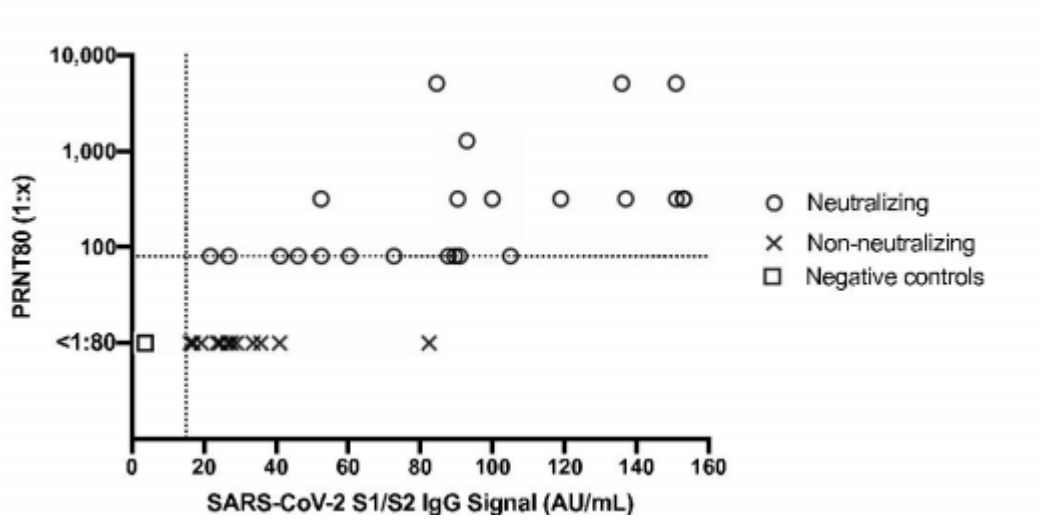

Figure 2. Receiver operator characteristic curve of the LIAISON SARS-CoV-2 S1/S2 IgG immunoassay signal value for the detection of NAbs. The area under the curve is 0.924.