Research Article

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SARS-CoV-2 Neutralizing Antibody Presence in Infected and Vaccinated Patients

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ABSTRACT

As of January 2022, it is estimated that over 60 million individuals have been infected by the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in the United States, and over 300 million individuals worldwide [1]. These staggering numbers have burdened physicians and scientists with the monstrous task of treating infected patients whose symptoms range in severity from asymptomatic to life threatening, while concurrently investigating the novel disease's biology and immune responses to infection and vaccination. While emerging literature has helped illuminate aspects of physiological immune responses to coronavirus disease 19 (COVID-19), the relationship between symptom severity and humoral immune response remains poorly understood. Additionally, the impact of this variability on the development of protective immune responses and the role of antibodies in disease is unclear, which has raised questions on the difference between neutralizing antibodies in those vaccinated and those unvaccinated. Our study aims to explore neutralizing antibody presentation against COVID-19 in groups of individuals with different histories of SARS-CoV-2 exposure and vaccination status. Thus, our findings will help further the understanding of humoral immunity as it relates to COVID-19.

***Conclusion**: There is a relation between total antibodies and neutralizing antibodies in people with various COVID histories.*

Introduction

SARS-CoV-2 is the virus that causes COVID-19, an acute respiratory disease that resulted in a pandemic outbreak in 2020. The overwhelming infection rates and devastating fatalities catalyzed an urgent need for scientists and physicians to clarify immunological responses that mediate protection against SARS-CoV-2 and offer protective immunity against the COVID-19 disease. The rapid necessity for treatment and protection has led the scientific community to partly focus on antibody response and exploring the ability to protect the body from disease via antibodies. Antibodies (Abs) are a key component of humoral immunity, which are produced by B cells after viral infection or vaccination and provide a first line of defense against subsequent exposures. Neutralizing Antibodies (NAbs) differ from non-neutralizing antibodies by binding to the pathogen and preventing it from interacting with the host cell, virtually making it ineffective. Antibodies to the novel coronavirus recognize the virus' external protein structure, and binds to the surface of the virus, preventing attachment to the host cell. Among the structural proteins of SARS-CoV-2, the spike (S) protein is the main target of human NAbs.

The S protein belongs to the class I trimeric fusion membrane protein and mediates virus entry via its receptor-binding domain (S-RBD), binding to the host receptor angiotensin-converting enzyme 2 (ACE2). Since COVID 19 is a disease with symptoms ranging in severity from asymptomatic to life-threatening, understanding how the body reacts to SARS-CoV-2 is critical in developing therapy and preventative protection. However, the relationship between symptom severity and humoral immune response is poorly understood, and the impact of this variability on the development of protective immune responses and the role of antibodies in disease is unclear. The emerging importance of using SARS-CoV-2 Abs to slow the spread and devastation of COVID-19, has allowed us to extensively research fighting the disease and gain valuable insight regarding immune response to infection and vaccination. Studies have shown that non-human primates (NHP) exposed to SARS-CoV-2 have antibody responses and are immune to reinfection [2]. Protection against SARS-CoV-2 is positively correlated with the development of high titers of neutralizing antibodies [3], and passive transfer of convalescent sera prevents infection enhancing the crucial role of antibodies in mediating protection against viral infection [4]. These preliminary findings spearheaded the effort to use Abs to develop therapy and a vaccine for COVID-19.

Prior to vaccine development, the importance of using Abs for infected individuals prompted physicians to use monoclonal antibodies or Convalescent plasma as treatment patients with COVID-19. The vaccine has offered protection to SARS-CoV-2 by introducing mRNA those codes for antibodies against SARS-CoV-2 via spike protein. The cell reads the mRNA and makes spike proteins, allowing the body's immunity to recognize the spike protein and produce antibodies against the spike protein. Because immune response differs from individual to individual, testing the level of neutralizing antibodies offer a more clear and direct understanding of one's protection against and readiness to fight off the SARS-CoV-2 virus. This focus on using SARS-CoV-2 Abs as a preventative measure has shown serum-neutralizing antibodies rapidly appear after SARS-CoV-2 infection and vaccination and are maintained for several months [5-9].

The development of antibody protection during SARS-CoV-2 infection has remained a pressing concern for public health and vaccine development. However, the protection quality from natural immunity versus vaccine immunity has stirred debates that fuel vaccine hesitancy and questions about the need for vaccination and boosters. In the present study, we further illuminate the relationship between total antibodies and neutralizing antibodies in people with various COVID histories. Because immune response differs from individual to individual, testing the level of neutralizing antibodies offer a more clear and direct understanding of one's protection against and readiness to fight off the SARS-CoV-2 virus. Additionally, comparing NAbs in individuals with differing histories of SARS-CoV-2 vaccination and infection history, we can better understand the different immune responsivity to the virus.

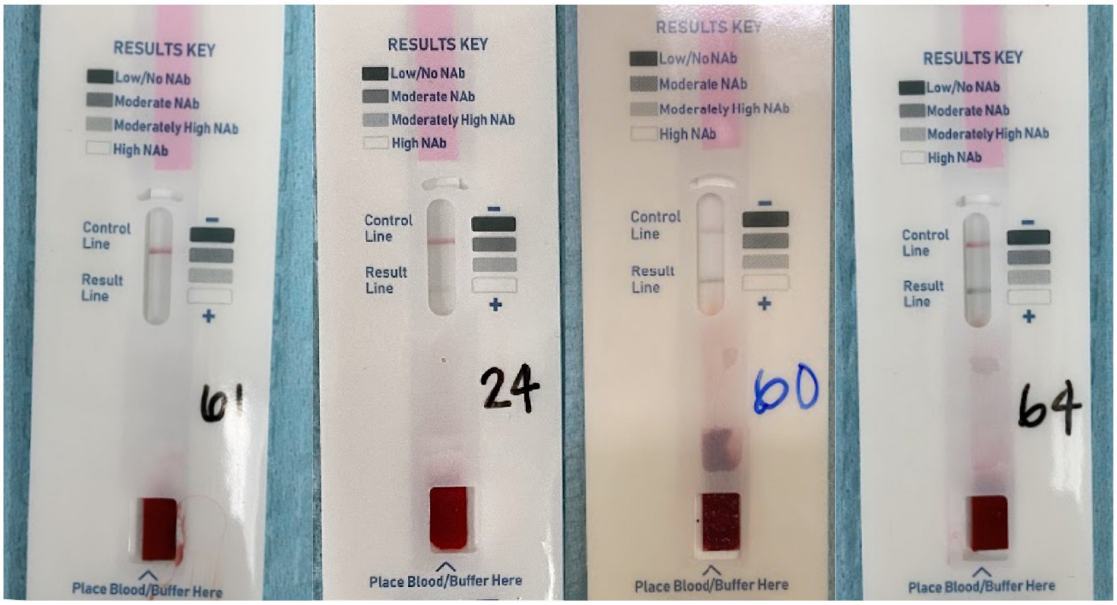
Material and Methods

To compare NAbs based on vaccination and infection history, subjects were divided into 4 groups for analysis: unvaccinated without disease history (Group 1), unvaccinated with disease history (Group 2), vaccinated without disease history (Group 3), and vaccinated with disease history (Group 4). After discussing the process and purpose of this study with participants, serum samples were obtained by centrifuging whole blood from 103 participants to assess neutralizing antibody activity against the SARS-CoV-2 virus. Testing for neutralizing antibodies were performed by directly using the plasma/EDTA extracted from the whole blood samples. Additionally, in patients who had trouble obtaining enough blood for analysis due to age or health factors, a Dry Blood Spot (DBS) analysis was performed. To obtain viable results via DBS, the blood spot was placed on a UDX100 card and 175uL of whole blood was added to the square application area (Figure 2) at the base of the card. After preparing the samples for analysis, two different methods for NAb analysis were performed. The first test utilized was the COVID-19 Neutralizing Antibody Rapid Test, which is a relatively new and growing procedure, to ensure test efficacy by matching results with total COVID-19 antibodies. For the rapid test, 15uL of whole blood was collected and filled to the designated line in the cassette (Figure 1), with 3 drops of the running buffer added fifteen seconds later. In order for the result to be valid, we ensured the control line appeared bold. Absence of the red control line after the mandatory ten-minute waiting time signified an invalid result. Additionally, we made sure not to interpret the results after 30 or more minutes had passed. To read the test, we compared the result line color to the results key provided on the top of the COVID-19 Neutralizing Antibody Rapid Test cassette (Figure 1). Because no exact NAb quantity is given for the rapid test, we categorized NAb presence by the boldness of the line to demonstrate high NAbs, moderate NAbs, low NAbs, or no NAbs. Second, we used a real-time PCR machine, Abbott Alinity, which produced a quantitative result, reported as AU/mL (absorbance unit per mL). To measure the total COVID-19 antibodies we used the Alinity instrument from Abbott in conjunction with the AdviseDx SARS Cov-2 IgG II calibrator and reagent kit (reference number 06S6101 and 06S6120, respectively). Core Laboratory at Abbott is located in Abbott Park, at 100 Abbott Park Rd North Chicago, Illinois.

This procedure takes much longer and is much more expensive, making it less accessible to the public and is unable to differentiate between total covid antibodies from neutralizing antibodies. Thus, using the neutralizing antibody rapid test in conjunction with the Abbott Alinity machine ensured results to be analyzed in terms of NAb quantity.

Results

This study found the total SARS-CoV-2 antibodies for 103 samples using the Alinity instrument from Abbott, 97 samples from plasma and 6 using DBS extraction. The samples collected via DBS rendered accurate results on par with the samples collected via venipuncture. Samples ran on the Alinity machine, provided a



61 Result: 0 (High NAb) 24 Result: *** (Moderately High NAb) 60 Result: **(Modeate NAb) 64 Result: * (Low/No NAb)

Figure 1. Varying COVID19 Neutralizing Antibody Rapid Test Results

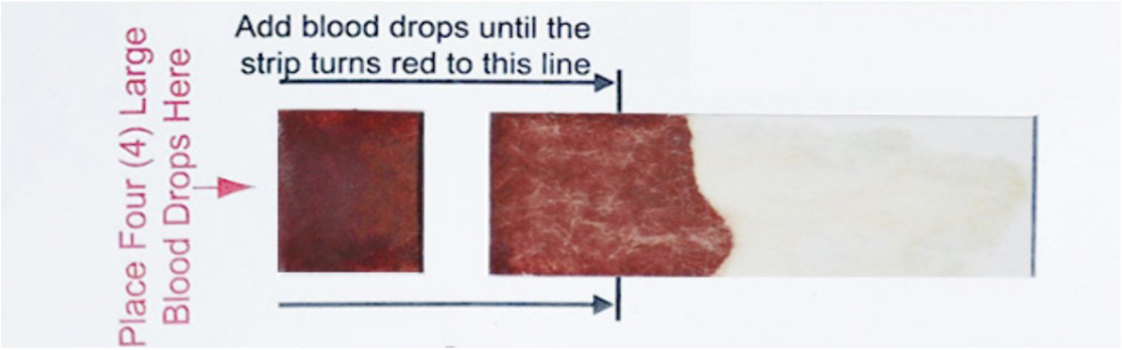


Figure 2. UDX100 Dry Blood Spot Card

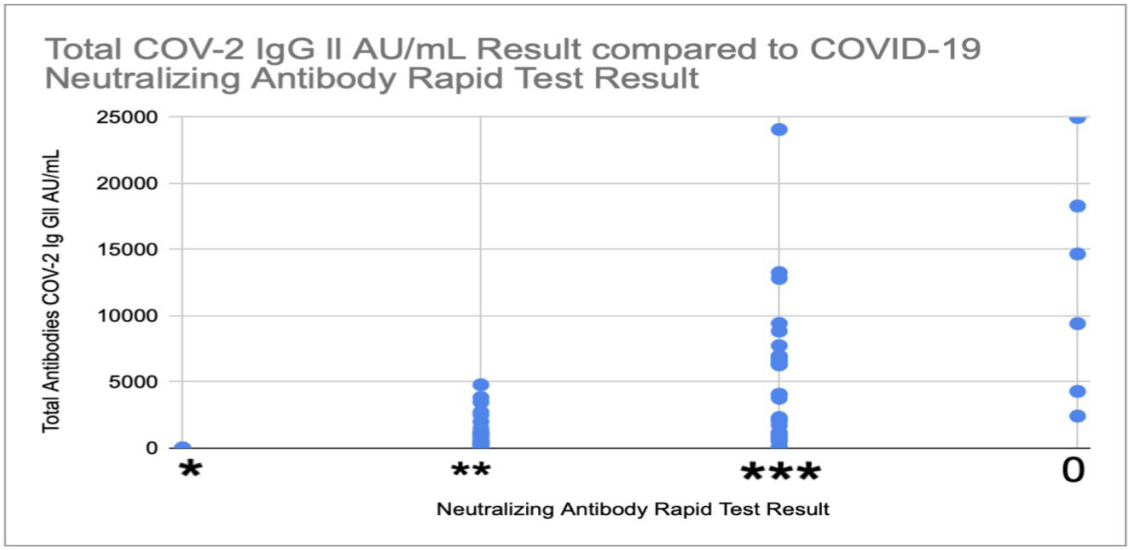


Figure 3: Total COV2 IgGII/AU/mL compared to the samples Neutralizing Antibody Rapid Result. For neutralizing antibodies * represents the lowest finding, ** represents moderate levels, ** is moderately high neutralizing antibodies, and 0 is high neutralizing antibodies. For more details see the COVID-19 Neutralizing Antibody Rapid Test in the Materials section.

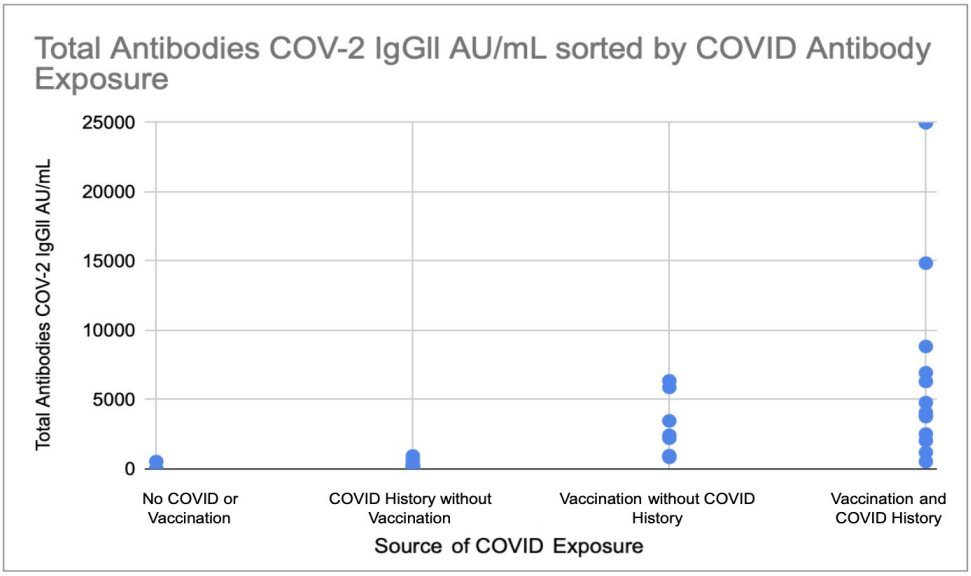


Figure 4: Total Antibodies COV2 IgGII/AU/mL sorted by Sources of COVID Antibody indicates a strong correlation between vaccination and increased antibodies

COVID Infection History Only, No Vaccine	
Sample Number	Final Result COV-2 IgGII AU/mL
1	6.5
2	934
3	53.2
7	84.6
8	262
11	654.4
23	385.9
58	267
94	0
96	65.4
COVID Infection History and Fully Vaccinated	
Sample Number	Final Result COV-2 IgGII AU/mL
4	2520.3
10	14849.7
20	3831.3
24	4073.8
32	528.8
37	25000
38	2033.4
47	8837.7
52	3774.3
54	4784.2
55	25000
56	1186.8
57	25000
59	25000
69	6308.7
74	6938.922

Table 1:

possible total range between 0 to 25,000 COV-2 IgGll AU/mL. Of the 103 samples, 89 were used on the COVID-19 Neutralizing Antibody Rapid Test. On the COVID-19 Neutralizing Antibody Rapid Test, 7 out of 89 (7.87%) yielded a low NAb result, 26 out of 89 (29.21%) yielded a moderate NAb result, 36 out of 89 (40.45%) yielded a moderate high NAb result, and 10 out of 89 (11.24%) yielded a high NAb result of the study participants, there were varying percentages, which fell into the four aforementioned groups of exposure to COVID antibodies. Patients in Group 1, or with no COVID infection or vaccine history, compiled 8.6% of participants and had an average antibody count of 177.1 COV-2 IgGll AU/mL. In Group 2, or participants who had a COVID infection but no vaccine history, encompassed 28.6% of total participants, and had an average antibody count of 271.3 COV-2 IgGll AU/mL. 45.7 percent of the total participants were placed in Group 3, and had both experienced COVID infection and had vaccine history; this group saw an average antibody count of 9979.25 COV-2 IgGll AU/mL. In Group 4, or the participants who were fully vaccinated with no previous covid infection, represented 17.1 percent of participants, and their average antibodies count was 3162.61 COV-2 IgGll AU/mL. All of this data is represented in Table 1 and 2. The group, which had the highest overall NAbs, were those in Group 3, who had both been vaccinated and had COVID Infection history, with an antibody count ranging from 538 to 25000 AU/mL. The discrepancy between these values are due to the varying gap in time between antibody production and study data collection.

No COVID Infection History, Fully Vaccinated	
Sample Number	Final Result COV-2 IgGll AU/mL
6	832.7
12	960.6
21	3466.2
27	6362.9
17	2421.9
15	2214.6
97	5879.4
No COVID Infection History or Vaccine	
Sample Number	Final Result COV-2 IgGll AU/mL
5	531.3
19	0
51	0

Table 2:

This study also collected voluntary information about demographic details including gender and race. The gender data features 35 people, 21 male and 14 female. The race results feature data for 16 Hispanic/Latino people, 2 Black people, 13 White people, and 3 Asian people. Additionally, the data from this experiment supports the conclusion that using the DBS for blood sample collection and plasma extraction are both accurate methods and render viable results.

Discussion

On average, the participants with the highest antibody levels were those in Group 3, or those that had both been vaccinated and had COVID disease history. This suggests a strong positive correlation with both actions leading to increased immunity, reaffirmed by the visual pattern possible to discern when data is sorted based on Antibody level. As seen in Figure 3, there is a discernible trend that as total antibodies increase, so too does a sample's total neutralizing antibodies. A key comparison between Groups 2 and 4, or fully vaccinated with disease history and unvaccinated with disease history, further illustrate the difference in antibody count based on vaccination. Group 2 participants on average had more antibodies than participants who had only had previous COVID-19 infection and had not been vaccinated. An important original consideration was the impact of the period between when a patient had COVID and when a patient donated blood for the study. However, while there was a general trend that those who had COVID and no vaccine had the disease earlier than those that had also been vaccinated, even patients who had the disease a month before sample extraction still had fewer than 1000 antibodies, much lower than those who received COVID and the vaccine in the same time frame.

These results (Figure 4) help validate recent studies, which suggest vaccination provides greater immunity than COVID disease exposure. For example, one study compared the neutralizing-antibody response to 4 variants in infected and vaccinated individuals to determine how mutations within the spike protein are associated with virus neutralization. By being, vaccinated patients have a greater antibody based immunity than those who just have COVID disease history. Unfortunately, this study has less statistical significance standing on its own because of the smaller sample size. In order to further confirm this research, more studies should be conducted with larger sample sizes.

A notable patient example is participant 5. When originally interviewed, this patient remarked that they had not had COVID or been vaccinated. However, after receiving their results of 531.3 COV-2 IgGll AU/mL, we inquired further about their disease history. When the participant asked again revealed they had possibly been exposed a few months back but never were tested so could not be sure of disease history and therefore originally responded with no disease history. Given their data however, it seems likely that participant 5 did indeed have COVID, but simply was not tested during the appropriate time and was asymptomatic.

This study also made efforts to analyze the impact of demographic identifiers of participants in order to draw peripheral conclusions. One demographic factor to consider is ethnicity. 35 of the studies participants chose to participate in the additional demographic survey. This survey asked participants to self-identify their race as either Hispanic/Latino, White, Black, or Asian. The category of “Other” was also included but upon further inquiry, the participants who identify as Middle Eastern agreed to be included under the racial umbrella of White. Of these participants, there were 16 Hispanic/Latino, 2 Black, 13 White, and 3 Asian subjects.

These groups had average antibodies of 7195.25 COV-2 IgGll AU/mL, 12730.1 COV-2 IgGll AU/mL, 3502.8 COV-2 IgGll AU/mL, and 1984.03 COV-2 IgGll AU/mL respectively. While this data is important, it is unreasonable to draw conclusions on an unevenly distributed sample group. This is especially true of our dataset concerning black people considering there are only two data points available for reference. Future studies should make an effort to specifically analyze the impact of ethnicity on a person's COVID antibody levels.

Another important demographic possible to analyze is gender. 35 of the participants' gender was self-identified. Of these participants, 21 of them were male and 14 were female. The group of females had a higher count of antibodies with an average of 7052.443467 COV-2 IgGll AU/mL while the male group averaged 4863.503 COV-2 IgGll AU/mL. However, with this small of the sample size we are unable to draw a conclusion that shows statistical significance. In order to be fully certain of this correlation additional data must be collected.

Although the results obtained in the present study demonstrate those who were vaccinated and had history of COVID-19 achieved the highest possible immunity against SARS-CoV-2, there are several limitations to this study that hindered showing statistical significance. First, given the relatively small sample size of this study it is difficult to draw any conclusions with a high level of statistical certainty. In order to further confirm that vaccination provides a more robust antibody immunity to SARS-CoV-2, more studies should be conducted with larger sample sizes. Not only would this allow us to further illustrate the impact of vaccination or infection history on NAb production, but it would also enable us to gather more data on population demographics as it pertains to NAb presence in varying ethnic groups and populations. Second, this study could be improved by longitudinally evaluating NAb presence within each group.

While these findings are promising and highly suggestive of a link between vaccination and higher levels of antibodies, further studies should examine this correlation in relationship to a longitudinal study by collecting data from the same participants 6 months later. This data would help highlight the longer-term effects of immunity via vaccination compared to immunity via direct exposure to the disease. Another interesting possible future study would be to take this data and analyze what ethnic or gender groups tend to be more likely to contract COVID and which groups are more likely to get vaccinated. Using this data more effective public health measures could be implemented to help the demographics, which have lower vaccination rates, and higher disease history numbers get the access they need to necessary COVID services.

References

1. Deng W, Bao L, Liu J, et al. Primary exposure to SARS-CoV-2 protects against reinfection in rhesus macaques. Science. 2020; 369: 818-823.
2. Mercado NB, Zahn R, Wegmann F, et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature. 2020; 586: 583-588.
3. Hassan AO, Case JB, Winkler ES, et al. A SARS-CoV-2 Infection Model in Mice Demonstrates Protection by Neutralizing Antibodies. Cell. 2020; 182: 744-753.e4.
4. Chen P, Nirula A, Heller B, et al. SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with Covid-19. N Engl J Med. 2021; 384: 229-237.
5. Ju B, Zhang Q, Ge J, et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. Nature. 2020; 584: 115-119.
6. Yan R, Zhang Y, Li Y, et al. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 2020; 367: 1444-1448.
7. Guo Y, Huang L, Zhang G, et al. A SARS-CoV-2 neutralizing antibody with extensive Spike binding coverage and modified for optimal therapeutic outcomes. Nat Commun. 2021; 12: 2623.
8. Beyerstedt S, Casaro EB, Rangel EB. COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. Eur J Clin Microbiol Infect Dis. 2021; 40: 905-919.
9. Yuan M, Liu H, Wu NC, et al. Recognition of the SARS-CoV-2 receptor binding domain by neutralizing antibodies. Biochemical and Biophysical Research Communications. 2021; 538: 192-203.