Longitudinal SARS-CoV-2 Vaccine Antibody Responses and Identification of Vaccine Breakthrough Infections Among Healthcare Workers Using Nucleocapsid IgG

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ABSTRACT

Background: Long-term studies of vaccine recipients are necessary to understand SARS-CoV-2 antibody durability, assess the impact of boosters on antibody levels, and protection from infection. The identification of vaccine breakthrough infections among fully vaccinated populations will be important in understanding vaccine efficacy and SARS-CoV-2 vaccine escape capacity.

Methods: SARS-CoV-2 Spike-RBD (S) and Nucleocapsid (N) IgG levels were measured in a longitudinal study of 1000 Chicago healthcare workers who were infection-naïve or previously-infected and then vaccinated. Changes in S and N IgG were followed up to 14 months and vaccine breakthrough infections were identified by increasing N IgG.

Results: SARS-CoV-2 S IgG antibody levels among previously infected and previously non-infected individuals decreased steadily for 11 months after vaccination. Administration of a booster 8-11 months post-vaccination increased S IgG levels more than 2-fold beyond those observed after 2 doses resulting in indistinguishable S IgG levels between previously-infected and uninfected individuals. Increases in N IgG identified vaccine breakthrough infections and showed over 15% breakthrough infection rates during the Omicron wave starting in December 2021.

Conclusions: These results demonstrate SARS-CoV-2 antibody changes after vaccination and breakthrough infections and identify high levels of vaccine breakthrough infections during the Omicron wave based on N IgG increases.

Keywords: SAR-CoV-2, Vaccine, Booster, Antibodies, Breakthrough infection

BACKGROUND

The SARS-CoV-2 pandemic has shown few signs of slowing down and the emergence of variants with increased symptom severity (Delta[1]) and transmissibility (Omicron[2, 3]) have continued to fuel renewed waves of global infections. While vaccines against SARS-CoV-2 have proven to be instrumental in reducing symptom severity and mortality even among Delta and Omicron variants[4], important questions remain regarding the durability of vaccine protection, length of time that vaccine induced antibodies are detectable[5], and what impact prior infection may have on vaccine response. Furthermore, the recommendation for individuals to receive a booster dose raises questions about antibody responses, protection from severe infection, and antibody durability after booster administration[6]. Lastly, identifying vaccine breakthrough infections using Nucleocapsid (N) IgG in vaccinated populations will be important for estimating the prevalence of SARS-CoV-2 infections and will play a key role in identifying vaccine breakthrough infections[7].

Recent reports have highlighted the effect of "hybrid immunity" on SARS-CoV-2 vaccine responses, showing that previous infection leads to both higher antibody levels as well as neutralizing antibody titers in vaccinated individuals who were infected prior to vaccination[8-10]. This hybrid immunity difference was shown to persist at least 3-months after two vaccine doses and was normalized when naïve individuals were given a 3rd vaccine dose[9]. These studies focused on a small number of individuals (fewer than 100) and larger studies following individuals for longer periods of time will be important to confirm the impact of hybrid immunity on vaccine driven antibody responses, especially in light of new vaccine evading variants.

Previous SARS-CoV-2 antibody studies have primarily focused on quantitating Spike (S) IgG antibody levels after vaccination or boost[11, 12] or on N IgG as part of seroprevalence studies[13]. Studies that utilize both S and N serology are needed to understand vaccine antibody kinetics as well as identify vaccine breakthrough cases among fully vaccinated individuals.

In this study, we enrolled 1000 Rush University Medical Center healthcare workers who were fully vaccinated with Pfizer/bioNTech BNT162b2 and either previously infected with SARS-CoV-2 or were infection naïve. We report longitudinal S antibody levels collected at 3-month time intervals spanning 15 months and approximately 3-6 weeks after a booster dose. Finally, we used longitudinal changes in N antibody levels and self-reported positive tests to identify likely vaccine breakthrough infections and show that routine N antibody testing can be used to track SARS-CoV-2 incident infections among a fully vaccinated/boosted population.

MATERIALS AND METHODS

This study was designed to test blood antibody levels to the SARS-CoV-2 Spike (S) and Nucleocapsid (N) proteins in participants receiving an mRNA-based vaccine. Six (6) time points (1-month, 5-months, 8-months, 11-months, 14-months post full vaccination and approximately 3-6-weeks post booster) were collected. This study was approved by the Rush University Medical Center IRB. Study participants were Rush University Medical Center healthcare workers who enrolled between January and March 2021 and provided informed consent to provide up to 20 ml of venous blood from which plasma was isolated, deidentified, and shipped to Abbott Diagnostics (Abbott Park, IL) on dry ice for testing. Participants self-reported demographic data including age (years), race/ethnicity (B=Black, W=White, A=Asian, H=Hispanic), sex (Male/Female), and history of COVID-19 infection including symptoms and SARS-CoV-2 test results. In total, 1000 individuals were enrolled in the study. After removing participants who were self-reported to be immunocompromised (n=32), had received only one dose of vaccine (n=10), or did not return for following blood draws (n=21), 937 participants remained. Baseline screening, testing, and participant history questionnaires identified 805 participants with no evidence of prior SARS-CoV-2 infection and 132 individuals with evidence of previous SARS-CoV-2 infection. Due to scheduling conflicts/inability to return, timing of booster vaccination, or identified vaccine breakthrough infection,

934, 868, 583, 359, and 465 participant samples were available for testing at 1-month, 5-month, 8-month, 11-month, and 14-month timepoints, respectively. Post booster draws were available for 666 participants and were collected approximately 3-6 weeks after boost. Individuals who received a booster were analyzed separately from those who did not for the remaining timepoints of the study. At the 14-month time point, the participants who returned for antibody testing were similar to those who did not return with respect to age ± SD (45.3 ± 13.4, range 21 to 84 versus 40.4 ± 12.1, range 22 to 81), sex (79.7% female versus 74.1%), race (79.7% white versus 80.0%) and previous infection status (13.6% previously infected versus 14.4%).

SARS-CoV-2 antibody detection

All samples were run on an Abbott ARCHITECT™ *i*2000SR instrument and tested using the FDA EUA approved SARS-CoV-2 IgG and SARS-CoV-2 IgG II Quant assays according to the ARCHITECT operations manual and assay package insert instructions. Briefly, the SARS-CoV-2 IgG assay is an automated Chemiluminescent Microparticle Immunoassay (CMIA) used for the qualitative detection of IgG antibodies directed against the SARS-CoV-2 N protein. Assay results are measured in Relative Light Units (RLU) and reported as an Index value of the ratio of specimen to calibrator RLU signal (S/C). Index values ≥1.4 S/C indicate a SARS-CoV-2 IgG seropositive result with an overall specificity rate of 99.63% at the cutoff per package insert. For the identification of vaccine breakthrough infections using N IgG, an increase in SARS-CoV-2 IgG Index values of ≥0.8 over the baseline draw were used. An alternative cutoff of 0.8 Index was shown to have a specificity of 99.6% in an external study[14] and was further supported in additional studies[15, 16], particularly with regards to seroprevalence. The SARS-CoV-2 IgG II Quant assay is an automated CMIA used for the quantitative detection of IgG antibodies directed against the receptor binding domain (RBD) of the SARS-CoV-2 S-protein with assay results reported in arbitrary units (AU/mL). Assay calibration is performed using 6-point calibration referencing an internal reference

standard at each concentration level. Results <50.0 AU/mL are reported as negative and ≥50.0 reported as positive and results ≥40,000 AU/mL were diluted, retested, and corrected for dilution factor.

Statistical Analysis

Study participants (n=937) were grouped into two cohorts based on previous evidence of SARS-CoV-2 infection (previous positive PCR and/or positive SARS-CoV-2 N IgG at baseline). In total, 805 participants did not have evidence of previous SARS-CoV-2 infection and 132 participants did. Participants (n=167) who acquired a self-reported confirmed/suspected vaccine breakthrough infection during the study (self-reported positive SARS-CoV-2 test and/or N IgG increase ≥0.8 Index over baseline) were excluded from later timepoints and analyzed separately as part of the vaccine breakthrough cohort. December 1st, 2021, was used to define the start of the Omicron wave in the Chicago area based on increasing reported prevalence of sequenced Omicron infections. Data reported by the Chicago Department of Public Health indicates that Omicron variants were over 20% by December 4th 2021 and reached >95% of cases sequenced at Northwestern University by December 14th 2021[17]. All graphing and statistical analysis was conducted with GraphPad Prism Version 8.0.2. Specifically, Mann-Whitney U and Chi-square tests were used to assess differences in SARS-CoV-2 IgG and vaccine breakthrough levels between timepoints in the cohorts described above. Significance was defined as $p<0.05$ and exact values out to 3 decimal places are reported. Descriptive statistics such as median and interquartile ranges (IQR) were computed in GraphPad Prism. Age vs antibody response graphs including best-fit lines, 95% confidence intervals, R square, and slope *p* values were calculated using Prism's linear regression analysis.

RESULTS

In total, 1000 individuals were enrolled in the study. After removing participants who were self-reported to be immunocompromised (n=32), had received only one dose of vaccine (n=10), or did not return (n=21), 937 participants remained. Self-reported participant demographics are shown in Table 1 and participants were largely white (63.8%), female (72.9%), and had median (IQR) age of 41 (33-54) years (7.1% aged 65 and older). Evidence of previous SARS-CoV-2 infection was identified in 132 (14.1%) individuals. Quantitative Spike-RBD (S) antibody levels in study participants were measured at 1, 5, 8, 11, and 14-months following the administration of a second SARS-CoV-2 mRNA vaccine dose. In participants who received a 3rd (booster) dose, an additional draw was taken approximately 3-6 weeks later. Participants who received a booster during the study (n=666) were between 8-11 months after their second dose. Participants with a prior SARS-CoV-2 infection showed a non-significant correlation between age and S IgG levels 1-month after full vaccination (r=.17, *P*=.051) (Figure 1A). Participants without evidence of a previous SARS-CoV-2 infection showed a negative correlation between age and S IgG levels 1-month following full vaccination (r=-.23, *P*<.001) (Figure 1B).

Median (IQR) S antibody levels of the combined study participants were 10394 (6149-17413), 1568 (920-2579), 782 (478-1346), and 890 (423-11317) AU/mL at 1, 5, 8, and 11-months (without booster), respectively (Figure 2A). The steadily decreasing antibody levels were significantly different at each measured timepoint. In the 666 participants who received a booster, median (IQR) S antibody levels increased to 23255 (13756-35551) AU/mL which was more than 2-fold higher than the peak observed 1-month after the second dose (*P*<.001). Median (IQR) S antibody levels at 11-months in individuals who received a booster were 14443 (7871-29850) which dropped to 10711 (5105-28487) at 14-months (*P*=.005).

Breaking out the results by infection status at the beginning of the study showed higher median (IQR) 1-month S IgG levels in participants who had previously been infected (n=132) compared to those without prior infection (n=802) [18156 (9656-28821) and 9801 (5686-15520) AU/mL, *P*<.001, respectively] (Figure 2B). This was also observed at 5, 8, and 11-month (without a booster) timepoints (*P*<.001 for all timepoints). This difference disappeared 3-6 weeks after the administration of a booster dose, resulting in median (IQR) S antibody levels of [21694 (14006-33386) and 23546 (13755-35919) AU/mL, *P*=.402] in previously infected or uninfected respectively and continued at the 11 and 14-months w/boost timepoints (*P*=.331 and *P*=.821, respectively).

Individuals with vaccine breakthrough infections (n=167) were identified based on self-reporting of a positive SARS-CoV-2 test and/or an increase in N IgG of ≥0.8 Index over baseline. Analyzing SARS-CoV-2 S IgG in the subset of participants with vaccine breakthrough infections revealed significantly higher median (IQR) antibody levels [80809 (39108-108715) AU/mL] in individuals who were infected at the 14-month timepoint compared to those who had breakthrough infections at previous timepoints (Figure 2C). The percentage of vaccine breakthrough infections identified at each timepoint steadily increased from 1.7% at 5-months after full vaccination to 15% at the 14-month timepoint in individuals who received a booster. At the 11-month timepoint, there was a significant difference between the rate of breakthrough infections in individuals who had already received a booster, compared to those who did not [9.5% (n=29/304) vs 20% (11/55) respectively, chi-squared *P*=0.023] and median (IQR) antibody levels were significantly higher (*P*<.001) in the boosted [14443 (7871-29850)] compared to non-boosted [890 (423-11317)] populations (Figure 2A and 2C). Separating breakthrough infections based on previous infection status showed similar trends of higher S IgG levels at the 14-month (with booster) timepoint compared to individuals infected earlier in the study (Figure 2D). Infection naïve individuals with breakthrough infections identified at the 14-month draw had significantly higher S IgG levels than

5-, 8-, and 11-month w/boost breakthrough infections. Due to lower overall numbers, comparisons were not made between individuals with a previous infection.

Based on increasing numbers of vaccine breakthrough infections occurring over time, even among boosted individuals, we wondered whether the Omicron variant wave might be associated with the observations. Plotting the vaccine breakthrough sample draw date against the S IgG result showed higher S IgG levels and numbers of vaccine breakthrough infections after the introduction of the Omicron variant in the United States (Figure 3A). Median (IQR) S IgG levels of 58477 (34174-91747) AU/mL were significantly higher in vaccine breakthroughs that occurred after Dec 1st 2021 (n=134) compared with 26335 (3478-72067) AU/mL (*P*<.001) in vaccine breakthroughs (n=33) that occurred between June 1st 2021 and Nov 30th 2021 (Figure 3B). Since the introduction of vaccine boosters began shortly before the Omicron wave in the US, we wondered whether the higher measured S IgG levels may be in part explained by participants having recently received a booster prior to Omicron breakthrough infection. Median (IQR) S IgG levels of 49715 (32575-82660) in non-boosted vaccine breakthrough infections occurring after Dec 1st, 2021 (n=17) were significantly different (*P*=.005) from median (IQR) S IgG levels of 24444 (3387-38997) in non-boosted vaccine breakthrough infections occurring between June 1st and Nov 30th, 2021 (n=30) (Supplemental Figure 1). We did not observe any significant differences (*P*=.441) in S IgG levels between boosted (n=117) and non-boosted (n=17) individuals with breakthrough infections occurring after Dec 1st, 2021. N IgG Index levels and total infections also showed higher trends in individuals infected after Dec 1st, 2021, compared to those infected between June 1st and Nov 30th of 2021 (Figure 3C). Median (IQR) N IgG Index levels of 3.11 (2.03-4.56) Index were significantly higher in individuals infected after Dec 1st, 2021, compared with 2.06 (1.15-3.38) Index (*P*=.002) in vaccine breakthroughs occurring between June 1st and Nov 30th of 2021 (Figure 3D). The number of days between the N IgG positive blood draw and self-reported positive tests with respect to

pre- and post- Omicron wave infections showed no significant difference (*P*=.316), supporting the observation that the lower IgG levels in the pre-Omicron breakthrough infections were not due to antibody waning between positive test and N IgG blood draw (Supplemental Figure 2). Similarly, the group of participants who had breakthrough infections during the Omicron wave had similar demographics as participants who had breakthrough infections during pre-Omicron waves with respect to age mean ± SD (42.5 ± 12.6, range 22 to 70 versus 41.0 ± 13.0, range 23 to 66), sex (81.3% female versus 71.9%), and race (84.3% white versus 75.8%).

Among the individuals with a vaccine breakthrough infection, 108 (64.7%) reported both a positive test and had an increase in SARS-CoV-2 N IgG ≥0.8 Index over baseline. There were 41 (24.6%) individuals with SARS-CoV-2 N IgG increases ≥0.8 Index who did not test or had a negative test result. An additional 11 (6.6%) individuals reported a positive test but did not have N IgG increases ≥0.8 Index over baseline and 7 (4.2%) individuals did not respond. There was no significant difference in median (IQR) SARS-CoV-2 N IgG Index levels between individuals with or without a self-reported positive test [2.86 (1.72-4.51) and 2.88 (1.91-4.32), *P*=.608] (Figure 4A). Among individuals who self-reported a positive SARS-CoV-2 test, there was a significant difference between the median number of days between the group who had SARS-CoV-2 N IgG increases of ≥0.8 Index (37.5 days, n=108) at their next timepoint than those who did not (62 days, n=11, *P*=.026), suggesting antibody waning in the latter group as a potential explanation (Figure 4B). The timing of breakthroughs was also analyzed by separating into pre- and post-Omicron wave and is reported in Table 2. The majority, 80.2% (n=134), of breakthrough infections in the study occurred after November 2021 compared to 19.8% (n=33) which occurred between May and the end of November 2021. Positive SARS-CoV-2 tests were self-reported for 51.5% in the pre-Omicron waves and 76.1% during the Omicron wave, with an overall self-reported positive test rate of 71.3%.

Within the study, 41 (24.6%) breakthrough infections were identified on the basis of SARS-CoV-2 N IgG increases of ≥0.8 Index over baseline alone.

DISCUSSION

Here we have characterized longitudinal SARS-CoV-2 vaccine antibody levels in a large cohort of both previously infected and uninfected individuals with up to 14-months of follow-up and show significant amounts of antibody waning in both groups over time. Similar to our previous report[10] with a smaller cohort, we observed a negative correlation between age and S IgG among individuals who were not previously infected which was not observed in individuals who were previously infected. It remains unclear why prior infection may lead to higher antibody levels after vaccination in older adults and future studies may be useful to explore the potential immunological mechanisms behind this observation.

S antibody levels were consistently higher in previously infected individuals after vaccination, and we found that a 3rd mRNA dose given as a booster 8-11 months after primary vaccination increased median S IgG levels to more than 2-fold higher than initial vaccination in both groups. The results from our large study support previous findings showing that hybrid immunity leads to higher antibody levels after vaccination, and we further show this effect continues out to at least 11-months following the second vaccine dose. Furthermore, we similarly observed that a 3rd dose removed the difference in observed S IgG levels between previously infected or uninfected individuals and this effect persisted more than 3 months later. Combined, these results provide strong additional support for the presence of hybrid immunity in previously infected and vaccinated individuals and highlight the importance of booster administration in people who have already been infected with SARS-CoV-2 as well as those who have not yet been infected.

One key aspect of this study is the use of longitudinal S and N antibody testing which allowed us to identify and track individuals who had acquired a vaccine breakthrough infection by comparatively measuring increased levels of N IgG. Through self-reporting we identified that around 25 percent of individuals with breakthrough infections had never had a positive SARS-CoV-2 test. This could have important implications on studies measuring SARS-CoV-2 community transmission based on positive test rates alone and may suggest that the true number of SARS-CoV-2 infections might be up to 33% higher than positive tests alone would indicate. Importantly, increases in N IgG were observed in people who had previously been infected with SARS-CoV-2 and had been reinfected. Thus, routine longitudinal antibody testing using N IgG can be used to identify ongoing SARS-CoV-2 infections in fully vaccinated as well as previously infected populations, and even boosted individuals. This approach could possibly underestimate the number of breakthrough infections, particularly if longer periods (greater than 90 days) between sampling occur, given that in this study we observed 6.6% of individuals who did not have a N IgG increase yet reported a positive COVID-19 test. In a 2021 Belgium study, prior to Delta and Omicron waves, 22.2% of mild infections did not seroconvert and 2.6% of severe patients did not seroconvert to N IgG, and by 6-months after infection 61.1% of mild patients had seroreverted[18]. It is not clear why some individuals do not seroconvert after infection. Additionally, in our study a number of infections were not reported by self-testing, perhaps because individuals did not experience symptoms which would have caused a test to be performed. This has important implications for prevalence studies as new waves of highly infectious, immune evasive, variants such as Omicron[19] infect fully vaccinated and/or previously infected populations and may be circulating at higher than reported levels. Indeed, we observed a large increase in the number of vaccine breakthrough infections occurring in our cohort after Dec 1st, 2021, which directly coincided with the Omicron variant wave that occurred in the US during late 2021 and into 2022. These results highlight previous reports on the enhanced immune escape potential of the Omicron variant[20]. Given recent publications on Omicron's immune evasion

potential[21-24], we expect to identify more such cases in later study time points and as new BA.4 and BA.5 variants begin circulating.

Interestingly, we observed that both S and N antibody responses in vaccine breakthrough cases were higher during the Omicron wave when compared to previous variant waves. The increased level of S antibody could potentially be explained by the booster that most individuals received prior to Omicron, however 17 non-boosted individuals with Omicron wave breakthrough infections also showed higher S IgG levels compared to pre-Omicron breakthrough infections. Furthermore, the higher N IgG levels observed would also not be explained by booster doses. There was not a statistical difference in the number of days between the vaccine breakthrough serology draw and self-reported positive tests in the pre- and post-Omicron infection groups, suggesting the lower N IgG level observed in pre-Omicron infections is not due to timing differences. Because the assays that we used were developed to detect antibodies against the D614G variant, it is possible that the measured levels of S and N IgG are underestimated for Omicron infections due to the significant number of mutations occurring in this variant. Even if this is occurring, we still observed significantly higher S and N IgG levels in individuals with vaccine breakthrough infections during the Omicron wave. Additional studies using sequence confirmed infections will be useful to confirm these observations and identify potential biological explanations.

When combined, the results of this study highlight the utility of regular SARS-CoV-2 IgG testing for both S and N antibody levels and for tracking seroprevalence of SARS-CoV-2 infections in a fully vaccinated population. We also show much higher rates of vaccine breakthrough infections during the Omicron wave and that a considerable number of infections occurred without a positive test. These results should inform future studies that assess ongoing seroprevalence studies as Omicron and new variants continue to circulate. Limitations of the study include a lack of uniform timing of boosters and blood draws, reliance on self-reporting of positive test results, and a lack of sequence confirmed infections.

REFERENCES

1. Twohig KA, Nyberg T, Zaidi A, et al. Hospital admission and emergency care attendance risk for SARS-CoV-2 delta (B.1.617.2) compared with alpha (B.1.1.7) variants of concern: a cohort study. Lancet Infect Dis **2022**; 22:35-42.

2. Christensen PA, Olsen RJ, Long SW, et al. Signals of Significantly Increased Vaccine Breakthrough, Decreased Hospitalization Rates, and Less Severe Disease in Patients with Coronavirus Disease 2019 Caused by the Omicron Variant of Severe Acute Respiratory Syndrome Coronavirus 2 in Houston, Texas. Am J Pathol **2022**.

3. Agency UHS. SARS-CoV-2 variants of concern and variants under investigation in England: Technical briefing: Update on hospitalisation and vaccine effectiveness for Omicron VOC-21NOV-01 (B.1.1.529). 31 December 2021 ed, **2021**.

4. Levin EG, Lustig Y, Cohen C, et al. Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. N Engl J Med **2021**; 385:e84.

5. Goldberg Y, Mandel M, Bar-On YM, et al. Waning Immunity after the BNT162b2 Vaccine in Israel. N Engl J Med **2021**; 385:e85.

6. Bar-On YM, Goldberg Y, Mandel M, et al. Protection of BNT162b2 Vaccine Booster against Covid-19 in Israel. N Engl J Med **2021**; 385:1393-400.

7. Demmer RT, Baumgartner B, Wiggen TD, et al. Identification of Natural SARS-CoV-2 Infection in Seroprevalence Studies Among Vaccinated Populations. Mayo Clin Proc **2022**; 97:754-60.

8. Andreano E, Paciello I, Piccini G, et al. Hybrid immunity improves B cells and antibodies against SARS-CoV-2 variants. Nature **2021**; 600:530-5.

9. Rodda LB, Morawski PA, Pruner KB, et al. Imprinted SARS-CoV-2-specific memory lymphocytes define hybrid immunity. Cell **2022**; 185:1588-601.e14.

10. Moy JN, Anderson M, Shen X, et al. Neutralizing Antibody Activity to SARS-CoV-2 Delta (B.1.617.2) and Omicron (B.1.1.529) After One and Two Doses of BNT162b2 Vaccine in Infection-Naïve and Previously-Infected Individuals. J Infect Dis **2022**.

11. Sadarangani M, Marchant A, Kollmann TR. Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. Nat Rev Immunol **2021**; 21:475-84.

12. Anderson M, Stec M, Rewane A, Landay A, Cloherty G, Moy J. SARS-CoV-2 Antibody Responses in Infection-Naive or Previously Infected Individuals After 1 and 2 Doses of the BNT162b2 Vaccine. JAMA Netw Open **2021**; 4:e2119741.

13. Rosenberg ES, Tesoriero JM, Rosenthal EM, et al. Cumulative incidence and diagnosis of SARS-CoV-2 infection in New York. Ann Epidemiol **2020**; 48:23-9.e4.

14. Bryan A, Pepper G, Wener MH, et al. Performance Characteristics of the Abbott Architect SARS-CoV-2 IgG Assay and Seroprevalence in Boise, Idaho. J Clin Microbiol **2020**; 58.

15. Chew KL, Tan SS, Saw S, et al. Clinical evaluation of serological IgG antibody response on the Abbott Architect for established SARS-CoV-2 infection. Clin Microbiol Infect **2020**; 26:1256.e9-.e11.

16. Blain H, Tuaillon E, Gamon L, et al. Spike Antibody Levels of Nursing Home Residents With or Without Prior COVID-19 3 Weeks After a Single BNT162b2 Vaccine Dose. JAMA **2021**; 325:1898-9.

17. Health CDoP. Omicron in Chicago (updated January 7, 2022). Available at: https://www.chicago.gov/content/dam/city/sites/covid/reports/SARS-CoV-2_Omicron_public_20220107.pdf. Accessed 09/26/2022.

18. Van Elslande J, Oyaert M, Ailliet S, et al. Longitudinal follow-up of IgG anti-nucleocapsid antibodies in SARS-CoV-2 infected patients up to eight months after infection. J Clin Virol **2021**; 136:104765.

19. Fall A, Eldesouki RE, Sachithanandham J, et al. A Quick Displacement of the SARS-CoV-2 variant Delta with Omicron: Unprecedented Spike in COVID-19 Cases Associated with Fewer Admissions and Comparable Upper Respiratory Viral Loads. medRxiv **2022**.

20. Hoffmann M, Krüger N, Schulz S, et al. The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic. Cell **2022**; 185:447-56.e11.

21. Planas D, Saunders N, Maes P, et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature **2021**.

22. Dejnirattisai W, Huo J, Zhou D, et al. SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. Cell **2022**; 185:467-84.e15.

23. Cui Z, Liu P, Wang N, et al. Structural and functional characterizations of infectivity and immune evasion of SARS-CoV-2 Omicron. Cell **2022**.

24. Shah M, Woo HG. Omicron: A Heavily Mutated SARS-CoV-2 Variant Exhibits Stronger Binding to ACE2 and Potently Escapes Approved COVID-19 Therapeutic Antibodies. Front Immunol **2021**; 12:830527.

FIGURE LEGENDS

Figure 1. SARS-CoV-2 S IgG levels and age after full vaccination. Linear regression and correlation between Age and SARS-CoV-2 S IgG 1-month post full vaccination in previously infected (A) or previously uninfected (B) individuals. Solid and dotted lines indicate linear regression and 95% CI, respectively. GraphPad Prism's Spearman r correlation was used and *P*<.05 was considered significant.

Figure 2. SARS-CoV-2 Spike-RBD (S) IgG levels after vaccination and identification of vaccine breakthrough infections.

SARS-CoV-2 S IgG (AU/mL) levels measured in all participants (A) and in individuals with (+) or without (-) evidence of previous SARS-CoV-2 infection (B) at the indicated time points following full vaccination. SARS-CoV-2 S IgG antibody levels at each individual timepoint from all breakthrough infections (C) and individuals with (+) or without (-) evidence of previous SARS-CoV-2 infection (D) were analyzed separately. The number of participants analyzed in each timepoint and vaccine

breakthrough infections as a percent of total samples is reported under the graph. Blue circles represent the first timepoint where a participant was identified as having evidence of a vaccine breakthrough infection based on a self-reported test and/or Nucleocapsid IgG increase ≥0.8 Index over baseline. Mann-Whitney U tests were used for all statistical comparisons and *P*<.05 was considered significant.

Figure 3. SARS-CoV-2 Spike-RBD (S) and Nucleocapsid (N) IgG levels in vaccine breakthrough infections by draw date.

SARS-CoV-2 S IgG (AU/mL) (A) and SARS-CoV-2 N IgG (Index) (C) results from suspected/confirmed vaccine breakthrough infections plotted against the draw date of the sample. SARS-CoV-2 S IgG (AU/mL) (B) and SARS-CoV-2 N IgG (Index) (D) results from individuals infected between June 1st - Nov 30th 2021 were compared to individuals infected between Dec 1st 2021 - March 31st 2022. Solid black trendlines were calculated with GraphPad Prism. Vertical dotted lines indicate Dec 1st 2021 which was considered to be the start of the Omicron wave in the United States. Mann-Whitney U tests were used for all statistical comparisons and *P*<.05 was considered significant.

Figure 4. SARS-CoV-2 Nucleocapsid IgG levels in individuals with or without a self-reported positive test.

(A) SARS-CoV-2 Nucleocapsid (N) IgG (Index) results from individuals with identified vaccine breakthrough infections separated based on individuals with a self-reported positive test or no/negative test. (B) The number of days between self-reported positive test and N IgG blood draw was compared between individuals with both a positive test and N IgG increase to those with a positive test but without N IgG increase. An N IgG increase was considered positive if the measured

result was ≥0.8 Index over baseline testing. Mann-Whitney U tests were used for all statistical comparisons and *P*<.05 was considered significant.

Foot Note Page

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Author contributions. J.M. and A.L. conceived and designed the study. M.S. performed the RBD IgG Assays. J.M., M.A., A.G., T. M. M.B., R.T.S. and D.B. collected and analyzed the data. M.A., J.M., G.C. and A.L. wrote the manuscript with input from all authors.

Table 1: Participant demographics and SARS-CoV-2 Spike-RBD (S) IgG (AU/mL) results

	All Participants (n=937)	Previously Infected (n=132)	Naïve (n=805)
Age (years) Median (IQR)[a]			
Combined Participants	41 (33-54) n=921	37 (31-55) n=132	41 (33-53) n=789
Male (n=172; 18.4%)	44 (36-58) n=172	52 (35-62) n=18	44 (36-56) n=154
Female (n=683; 72.9%)	40 (32-53) n=676	36 (30-53) n=106	40 (33-53) n=570
Unknown (n=82; 8.8%)	38 (33-45) n=73	32 (31-36) n=8	39 (33-45) n=65
Race: n (%)			
White	598 (63.8)	87 (65.9)	511 (63.5)
Black	108 (11.5)	19 (14.4)	89 (11.1)
Asian	81 (8.6)	9 (6.8)	72 (8.9)
Native American	5 (0.5)	0 (0)	5 (.6)
Mixed Race	13 (1.4)	3 (2.3)	10 (1.2)
Unknown/Other	132 (14.1)	14 (10.6)	118 (14.7)
SARS-CoV-2 IgG S AU/mL Median (IQR):[b]			
1-month	10394 (6149-17413) N=934	18156 (9656-28821) N=132	9801 (5686-15520) N=802
5-month	1568 (920-2579) N=869	3026 (1582-6635) N=117	1448 (864-2342) N=752
8-month	782 (478-1346) N=583	1809 (931-4818) N=81	725 (450-1142) N=502
3-6 weeks post boost	23255 (13756-35551) N=666	21694 (14006-33386) N=91	23546 (13755-35919) N=575
11-month w/boost	14443 (7871-29850)	13022 (5559-25530)	14472 (7916-30360)

	N=304	N=32	N=272
11-month wo/boost	890 (423-11317)	6254 (1906-14256)	500 (387-3358)
	N=55	N=18	N=37
14-month w/boost	10711 (5105-28487)	11251 (5402-26929)	10619 (5078-28816)
	N=466	N=63	N=403

[a] Self-reported ages were not available for 16 individual (7 women and 9 of unknown gender).

[b] Not all participants were able to provide a sample at every timepoint.

Table 2: Timing of self-reported and/or N IgG identification of SARS-CoV-2 breakthrough infections

Breakthrough Infection Timing	**Self-reported Positive Test**	**Self-reported No or Negative Test**	**No Response**	**Total n (% of total)**
May 2021-Dec 2021 (pre-Omicron) n (% of Total)	17 (51.5)	12 (36.4)	4 (12.1)	**33** (19.8)
Dec 2021-May 2022 (post-Omicron) n (% of Total)	102 (76.1)	29 (21.6)	3 (2.2)	**134** (80.2)
May 2021-May 2022 (Total) n (% of Total)	119 (71.3)	41 (24.6)	7 (4.2)	**167** (100)

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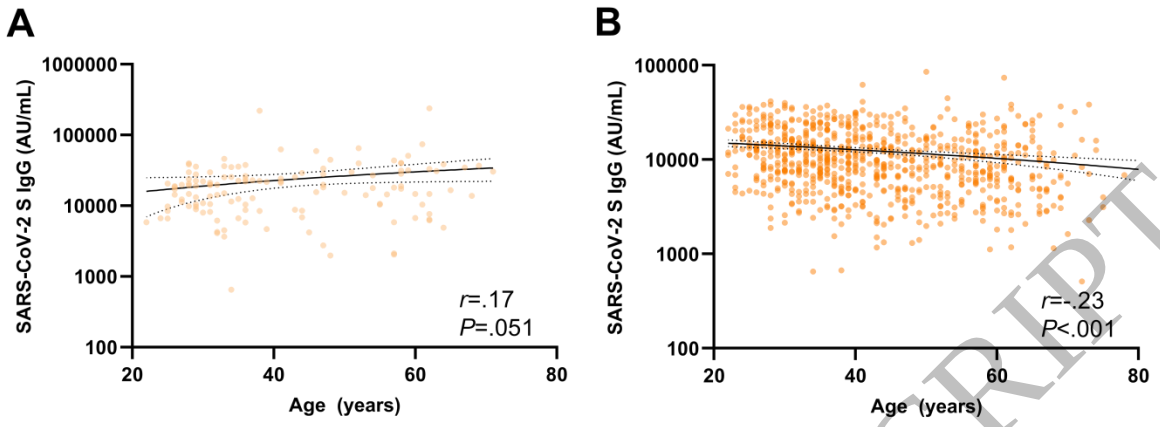


Figure 1
224x86 mm (x DPI)

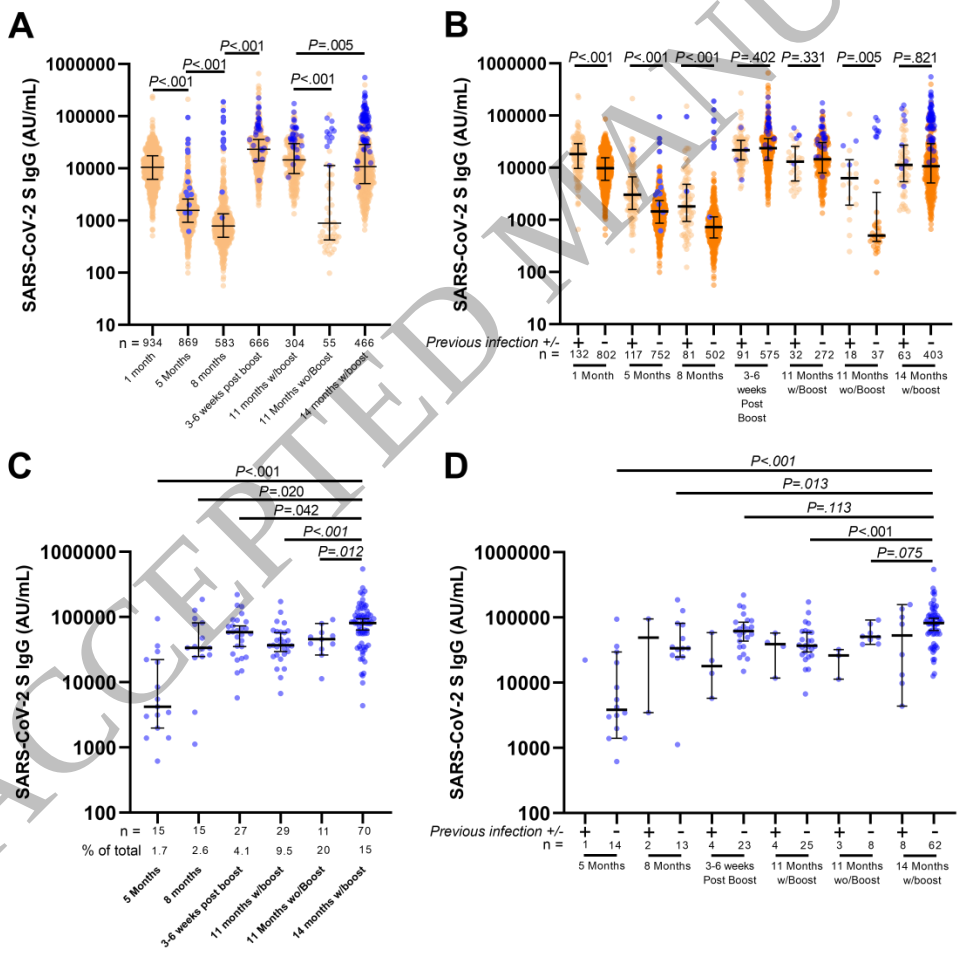


Figure 2
214x195 mm (x DPI)

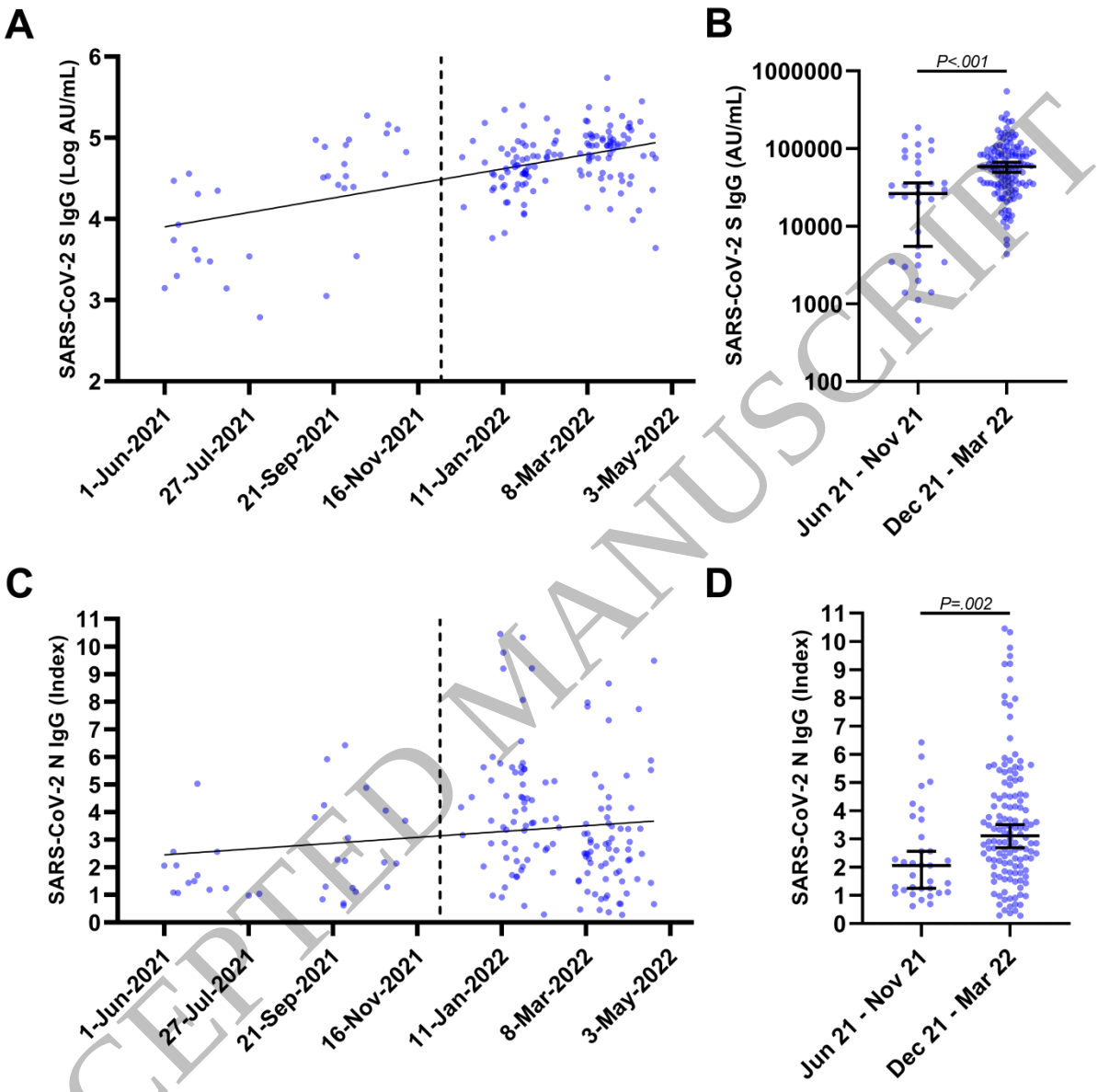
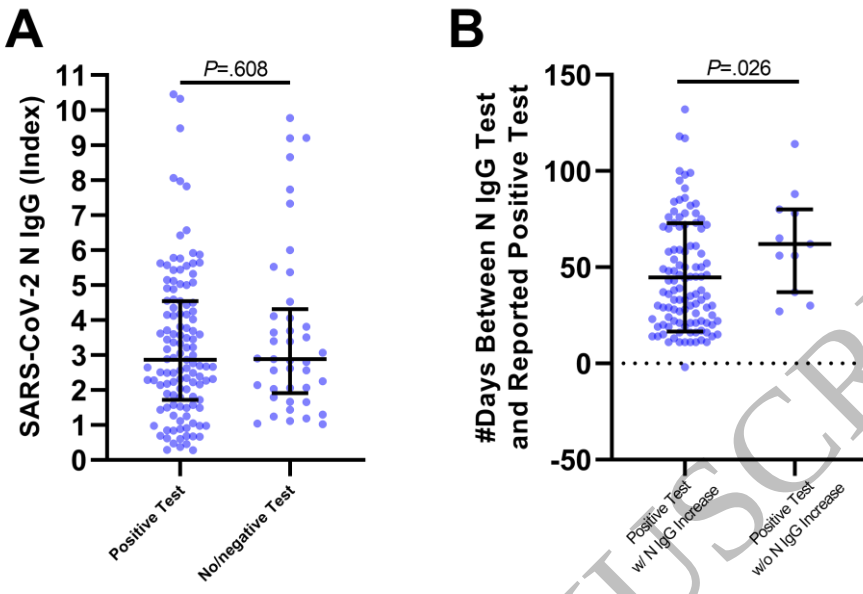


Figure 3
197x197 mm (x DPI)



1

2 **Figure 4**
3 **132x89 mm (x DPI)**