

Research Article

SARS-CoV-2 antibody response to third dose vaccination in a healthy cohort

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Abstract

Determining the extent of immunity induced by booster doses of COVID-19 vaccinations is crucial for informing recommendations for booster dose regimens as well as constant adjustments of immunization strategies amongst different groups of people within the population. The study involved 31 healthy volunteers (majority were healthcare professionals) who completed either vaccination course with Pfizer or Moderna mRNA vaccines and received a third dose of the vaccine. Here we report results on the evaluation of an antibody response to four different SARS-CoV-2 antigens: RBD, S1, S2 and nucleocapsid prior to third dose and two and four weeks after a booster vaccination. We detected a peak of high titers of antibodies after the third dose with a gradual decline after four weeks. No significant differences were seen between the two vaccines in terms of antibody response. There were no gender discrepancies between the two vaccines. Our results suggest that: third doses are necessary due to the emergence of different SARS-CoV-2 variants and postvaccination antibody testing continues to be essential in determining possible standardization of SARS-CoV-2 vaccine regimens.

Introduction

Severe acute respiratory syndrome coronavirus 2, also known as SARS-CoV-2 is the main cause of the coronavirus pandemic which began in 2019. In the last few years, vaccines have been shown to be effective against SARS-CoV-2 infection [1,2]. Production of antibodies starts within days to weeks after the first dose and studies have established the need for a second dose for improved efficacy [3,4]. Recent studies suggest that humoral immunity against SARS-CoV-2 associated with natural infection or vaccination may persist for several months however anti-viral antibodies will wane over time [5]. There are four major immunogenic proteins in SARS-CoV-2: spike, envelope, membrane and nucleocapsid which are encoded by their respective genes, *S*, *E*, *M* and *N* genes [6,7]. The *S* protein contains two subunits - S1 and S2, which mediate receptor binding and membrane fusion. Viral particles enter the host through a virus-host cell membrane fusion which requires a conformational change of the *S* protein. The receptor binding domain (RBD) (found on S1) binds to ACE2 (angiotensin-converting enzyme 2) on the host cell. This allows for a necessary conformational change of the S2

subunit permitting the membrane fusion [8,9]. Both natural infection of SARS-CoV-2 and vaccination allow for production and detection of anti-S1, S2, and RBD antibodies. After the second vaccine dose for most healthy volunteers, high levels of IgM, IgG anti-SARS-Cov-2 spike proteins *S* and RBD can be detected in circulation providing a surrogate measure of mRNA-vaccine induced immunity [10,11]. Natural infection is measured through anti-nucleocapsid antibodies which continues to serve as a marker for SARS-CoV-2 infection and it is not induced by spike protein-encoding vaccines [3].

The mRNA vaccine series for SARS-CoV-2 comprises of two doses for both Pfizer-BioNTech (30 µg, 0.3 mL each with three weeks apart) and Moderna (100 µg of 0.5 mL with one month apart) [12,13]. Initial doses of the vaccine showed elevated RBD IgG antibodies with steady titers at approximately three weeks with a significant increase after approximately seven days. After the second dose, levels of antibodies to the spike protein were increased at approximately five weeks, compared to that of 2 weeks - 3 weeks [14]. Similarly, other studies reported that amongst previously uninfected healthy people, anti-spike protein titers after one dose of the vaccine

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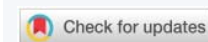
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were comparable to the peak of the anti-spike protein titers seen in those with natural infection without prior vaccination [15]. Additional studies also show agreement with results of an antibody response in the first vaccine dose in individuals with preexisting immunity which is equal to or even exceeds titers in naïve individuals after second vaccine doses [3,12].

There have been numerous attempts to standardize the quantification of the level of antibodies, however with variations in manufacturers, it is difficult to create such a standardized vaccine correlate of protection [16]. The goal of this study was to determine the change in the level of antibodies to RBD, S1, S2 and nucleocapsid SARS-CoV-2 antigens in healthy volunteers receiving their third dose of SARS-CoV-2 mRNA vaccines at two and four weeks following third dose.

Materials and methods

Study design and participants

Eligible participants were healthy individuals 18 years or older. There were a total of 31 participants who received two injections of one of the United States (US) emergency use authorized (EUA) mRNA vaccines (Pfizer/BioNTech or Moderna) and who would receive their third dose during the study. There were no enrolled participants who were subsequently excluded. The key exclusion criteria included: unwell at the time of recruitment, immunosuppression from disease or treatment, anemia, treatment for anemia or iron deficiency and known pregnancy. Written informed consent was obtained from each participant before enrollment in this study. Written approval of the study protocol and informed consent were obtained from the relevant independent ethics committee/Institutional Review Board (IRB) of the University of Pittsburgh (study #:21090015) and all work was performed in accordance with the Declaration of Helsinki. Blood samples were collected within the week prior to the third vaccine dose, two weeks after third dose and then four weeks after third dose. Length of time between second and third vaccine doses was a median 281 days (range: 200 days - 328 days).

Detection of virus-specific antibodies

Blood samples were collected into serum gel separator tubes and centrifuged after complete clotting at room temperature. Serum was collected from participants at the relevant time points and stored at -30 °C for up to four months before analysis. SARS-CoV-2 antibody assays were performed in the CLIA certified high-complexity clinical laboratories at the University of Pittsburgh Medical Center. We used the BioPlex 2200 CoV-2 IgG Panel (Bio-Rad laboratories, Inc., Hercules, CA) which is a multiplex assay for qualitative (IgG screening) and semi-quantitative (U/mL) detection of IgG class antibodies against RBD, S1, S2 and nucleocapsid proteins of SARS-CoV-2 virus in human serum and plasma. This assay is for research use only in the US but was run in accordance

with all local clinical laboratory requirements. The analytical measuring range for the semi-quantitative assay is 1 to 100 U/mL with a threshold of ≥ 10 U/mL being positive. There are onboard dilutions of 1:8, 1:16 and 1:32. Samples with levels higher than 3200 U/mL were manually diluted at 1:10 with the assay dilution buffer. Performance studies showed a specificity of 99.8% and overall sensitivity to 96.3% according to the manufacturer's instructions.

Statistical analysis

Data was analyzed using non-parametric *t* tests by Prism (version 9; GraphPad, San Diego, CA) for all analysis and data presentation. We considered *p* values of < 0.05 to be significant. The data are presented as means \pm the standard errors of the mean (SEM).

Results

A total of 31 healthy volunteers were included ($n = 31$). Of those, 29 were health care workers. 45% of these participants were males, 55% were females. Five out of 31 identified as Asian ethnically and 26 out of 31 identified as Caucasian. 25% of subjects received Pfizer mRNA vaccine and 75% received Moderna (Table 1). All participants received the same brand of vaccine for all three doses.

To assess the change in SARS-CoV-2 IgG following third vaccine dosing, we looked at antibody responses to nucleocapsid to control for possible confounding infection with SARS-CoV-2, and spike 1 (S1), spike 2 (S2) and the Receptor Binding Domain (RBD). After third dose vaccination, antibody levels for RBD, S1, and S2 were significantly increased at 2 weeks and overall remained elevated at 4 weeks (Figure 1A-D). Within this cohort we detected two participants who had an increase in nucleocapsid IgG levels between weeks 2 and 4 following their third vaccine dose, indicative of likely SARS-CoV-2 exposure following their third vaccine dose (Figure 1A). With the exclusion of these two participants, *p*-value remained the same ($p = 0.2500$) and was not significant. For both RBD and S1, the baseline serology had detectable antibodies and

Table 1: Demographic information.

Demographic Information	Number (%)
Gender	
Male	14 (45%)
Female	17 (55%)
Race	
Asian	5 (16%)
Caucasian	26 (84%)
Pre-booster vaccine	
Pfizer	8 (25%)
Moderna	23 (75%)
Vaccine type	
Pfizer	8 (25%)
Moderna	23 (75%)
Use of immunosuppressive drugs	0 (0%)
COVID-19 prior to booster	1 (3%)
Known COVID-19 exposure prior to booster	2 (6%)
Use of post-exposure prophylaxis monoclonal antibody therapy	0 (0%)

there was a significant increase 2 weeks post third dose ($p < 0.0001$) from baseline. For anti-RBD and S1 antibodies, between 2- and 4-weeks post third dose, titers were declining however aggregate analysis did not demonstrate a significant difference (p varied from 0.0502 - 0.0759) (Figures 1B,1C). For S2, most participants were negative for S2 antibodies at baseline. There was a significant increase between baseline and 2 weeks post third dose ($p < 0.0001$) with a significant waning of detectable antibodies by 4 weeks post third dose ($p < 0.0002$ week 2 compared to week 4). However, variation in the detection of S2 could be due to either an assay-specific or a physiologic response (Figure 1D). We noted that although there is no significant difference in aggregate analysis between 2 and 4 weeks for RBD/S1, for several participants there is a significant decrease in detectable IgG, while for others there is a delayed increase (Figure 2A-C). Particularly, values for RBD, S1 and S1 IgG antibodies did not fall below 1500 U/mL, 9650 U/mL and 175 U/mL respectively at 2 weeks post third dose, or 1530 U/mL, 820 U/mL and 55 U/mL respectively at 4 weeks post third dose demonstrating a persistence vaccine-induced antibody response.

One of the goals in this study was to assess possible variation between the two mRNA vaccines. We assessed RBD and S1 antibody levels and found no significant difference between the two vaccine types at 2 weeks and 4 weeks post third vaccine dose (Figure 3A,B). We calculated the difference between baseline and 2 weeks, as well as the difference between 2 and 4 weeks post third dose with both vaccine

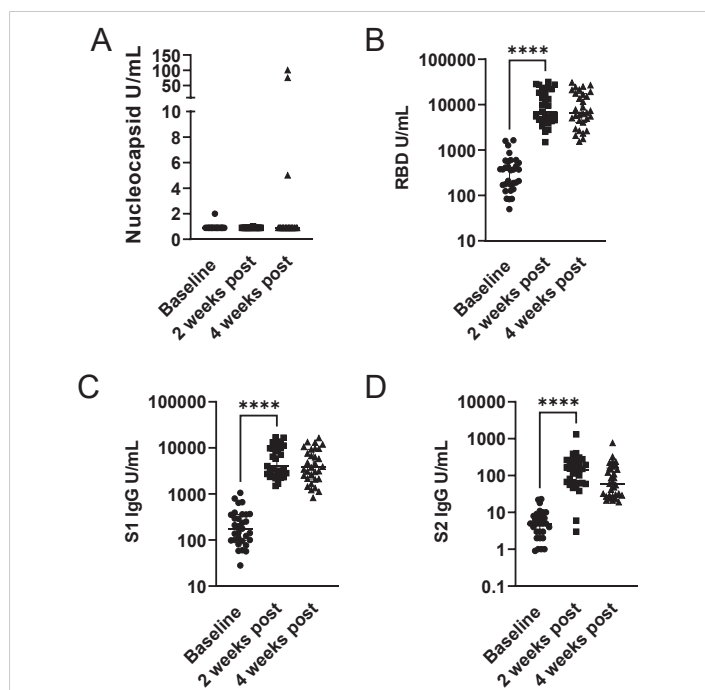


Figure 1: Antibody responses to third dose of mRNA vaccines. Blood specimens were collected from the cohort within one week before (0 weeks) and after the third dose of mRNA vaccines at two and four weeks. U/mL of binding antibodies are presented for each participant for (A) nucleocapsid IgG, (B) receptor binding domain IgG, (C) spike 1 subdomain IgG and (D) spike 2 subdomain IgG. Specimens are reactive at > 10 U/mL. Comparisons were performed by Wilcoxon and Mann-Whitney Rank Sum test, ****, p -value < 0.001 .

types to include both RBD and S1 antibodies and still found no difference between the antibody response to third dose vaccination. The p -value for RBD ranged from 0.5496 to 0.9824 and for S1 ranged from 0.3620 to 0.8078 (Figure 3A,B).

Lastly, we considered if there may be a difference between

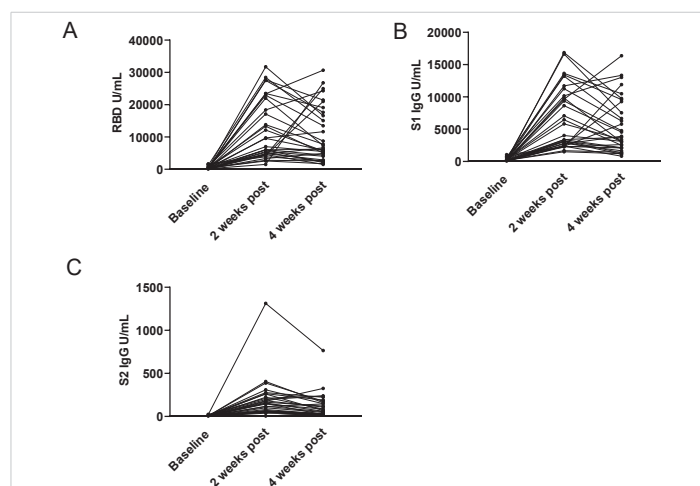


Figure 2: Temporal responses to third dose of mRNA vaccines. Blood specimens were collected from the cohort within one week before (0 weeks) and after the third dose of mRNA vaccines at two and four weeks. U/mL of binding antibodies are presented for each participant for (A) receptor binding domain IgG, (B) spike 1 subdomain IgG and (C) spike 2 subdomain IgG. Specimens are reactive at > 10 U/mL. Lines connect individual participants.

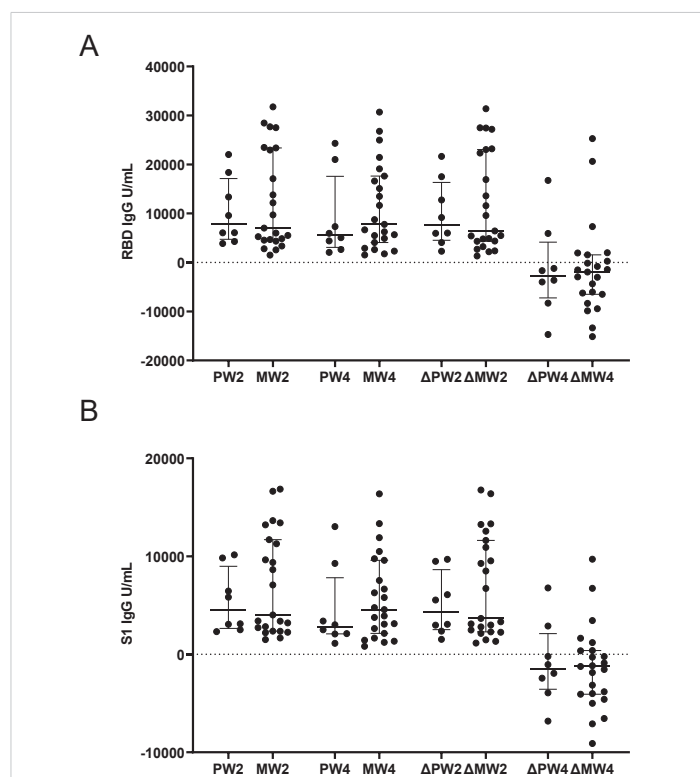


Figure 3: Comparison of Pfizer and Moderna antibody responses. Blood specimens were collected from the cohort within one week before (0 weeks) and after the third dose of mRNA vaccines at two and four weeks. U/mL of binding antibodies are presented for each participant for (A) receptor binding domain IgG, (B) spike 1 subdomain IgG. Abbreviations: Pfizer week 2 (PW2), Moderna week 2 (MW2), Pfizer week 4 (PW4), Moderna week 4 (MW4), change (Δ) for Pfizer (P) or Moderna (M) between weeks 2 and 0 (W2) or between weeks 4 and 2 (W4). Horizontal dashed line indicates positivity threshold (10 U/mL).

genders for both vaccines. We found no significant difference between males and females amongst vaccine types assessing RBD and S1 antibodies. p - value ranged from: RBD 0.0586 to 0.4683 and S1 ranged from: 0.1278 to 0.4683 (Figure 4A,B).

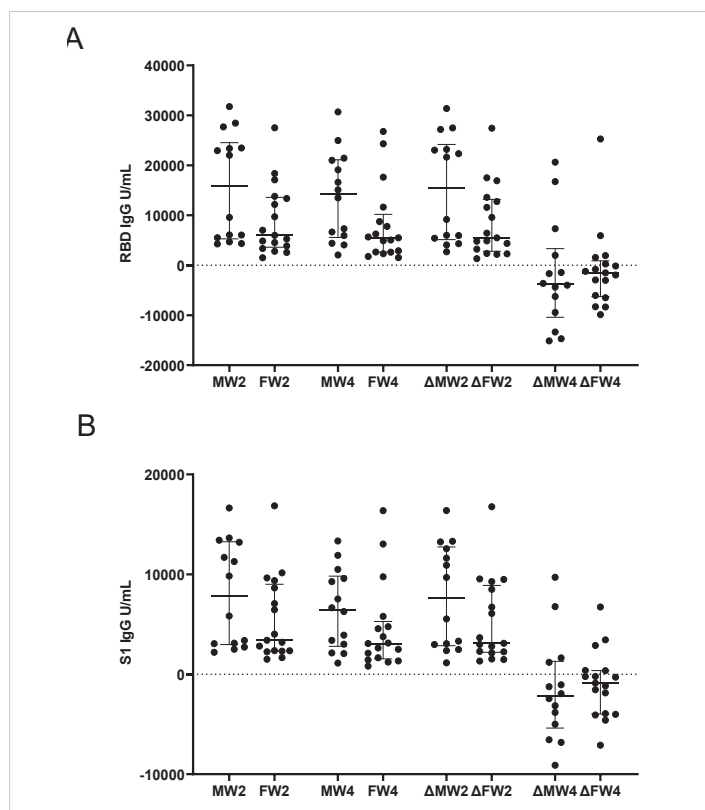


Figure 4: Comparison of male and female antibody responses. Blood specimens were collected from the cohort within one week before (0 weeks) and after the third dose of mRNA vaccines at two and four weeks. U/mL of binding antibodies are presented for each participant for (A) receptor binding domain IgG, (B) spike 1 subdomain IgG. Abbreviations: Male week 2 (MW2), Female week 2 (FW2), Male week 4 (MW4), Female week 4 (FW4), change (Δ) for Male (M) or Female (F) between weeks 2 and 0 (W2) or between weeks 4 and 2 (W4). Horizontal dashed line indicates positivity threshold (10 U/mL).

Discussion

Over the last two years, numerous SARS-CoV-2 variants have emerged and were classified as WHO variants of concern. Our study looked at the serological response of nucleocapsid, RBD, S1 and S2 antibodies after a booster vaccination of healthy donors. Specifically, we looked at the antibody response at two and four weeks post third doses. We found there was a vaccine-induced response in RBD, S1 and S2 IgG antibody levels by both vaccines, however there was no statistically significant difference between the Moderna and Pfizer vaccines. Similar studies also show an increase in antibody levels after third doses utilizing different assays [16,17].

After the third dose, we found, as expected, a significant increase in antibody levels for RBD, S1 and S2 ($p < 0.0001$ for two weeks post third dose and p value range from: 0.0002 to 0.0759 for four weeks post third dose). Our observations are concurrent with other studies assessing antibody levels post

third doses; several authors observed an antibody peak with values of antibody levels 2 to 3 times higher than baseline values. Interestingly, in our study, at 28 days after the third dose, there was a trend of decreasing antibody levels which was most prominent in S2 IgG antibody levels (minimum level of S2 antibody reported: 55 U/mL). Several other studies report similar findings in which while anti-S2 antibodies cross neutralize viral SARS-CoV-2 particles, they appear to decline earlier than anti-RBD antibodies thus decreasing its potency [18,19].

Our prior work using this assay during the primary vaccination series demonstrated average values of 5490 U/mL S1 and 15,300 U/mL RBD following second vaccination. Semiquantitative levels of the response from both doses are similar to the levels identified post third vaccination in our cohort for this specific study [3]. Similarly, there were no statistically significant differences in the potency of immune response to RBD protein after first and second doses of either vaccine.

The small sample size limits broad generalizations but provides a starting point for further work. Multicenter studies are needed to assess long-term vaccination plans as SARS-CoV-2 continues to change globally. Incorporation of nucleocapsid information allowed us to incorporate natural infection as an exclusion criterion in analyses, however, the exclusion of participants with nucleocapsid antibody positivity did not change the significance of the associated p values. Additionally, the follow-up time of our study was relatively short. Timely reporting for follow-up data is important in order to adjust immunization strategies in the context of the pandemic as variants emerge.

In conclusion, our serological data on third dose vaccine-induced antibodies, controlling for SARS-CoV-2 exposure through detection of anti-nucleocapsid antibodies, finds significant increases in antibody response to both mRNA vaccines with no significant difference between the vaccines. Numerous studies indicate the need for multicomponent vaccines which include and are not limited to the S protein as new variants may escape immune targeting [20-24]. Immunity progressively wanes through the course of two to four weeks for many participants implying that use of serology for epidemiologic studies or to assess vaccine response may need to consider time from vaccination or infection.

Author contributions

Concept and design: Michael Shurin, Sarah Wheeler, Simmi Patel.

Administrative support: Adam Anderson.

Provision and study materials or patients: Adam Anderson, Lisa Pinto, Sarah Wheeler, Michael Shurin and Simmi Patel.



Collection and assembly of data: Michael Shurin and Simmi Patel.

Data analysis and interpretation: Sarah Wheeler.

Manuscript writing: All authors.

Final approval of manuscript: All authors.

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We confirm that all relevant ethical guidelines have been followed, and all necessary IRB and/or ethics committee approvals have been obtained. Bio-Rad Laboratories had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Ethical statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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