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Response kinetics of IgG antibodies against SARS-CoV-2 targets: comparing vaccinated and non-vaccinated US clinical patients through linear analysis of Binding Antibody Units

By

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Degree to be awarded: Master of Public Health

Global Epidemiology

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Abstract

Response kinetics of IgG antibodies against SARS-CoV-2 targets: comparing vaccinated and non-vaccinated US clinical patients through linear analysis of Binding Antibody Units

By Ryan Sandford

This study addresses the importance of measuring antibody levels in the blood to understand SARS-CoV-2 infections and immune responses. Detecting present and past infections using serological data is still evolving, and there is a need for more consistent quantification of antibodies across studies and populations. Standardization of lab protocols and assays using World Health Organization (WHO) ratios and measurements is being pursued, but changes in variant prevalence and repeated waves of infections can complicate scientific determinations of infection and antibody status. This study was sourced from the FluVE Network and CDC Cares Act funding and used cross-sectional data from seven sentinel sites in the US. This study focused on a standardized assay for quantitative detection of SARS-CoV-2 antibodies to understand current patterns related to antibody responses. Blood was collected when patients presented for clinal care with respiratory symptoms, and a subset also had blood collected during the convalescent phase (paired collection). A 7plex assay was used to analyze IgG antibodies to the Receptor Binding Domain (RBD) and Nucleocapsid Proteins (NP) for analysis of SARS-CoV-2 antibody response by health condition, vaccination status, age, sex, and race. In the subset of the sample with a convalescent collection, anti-RBD and anti-NP antibody levels at baseline were assessed as predictors of antibody change over time. My thesis examined the relationship between COVID-19 vaccine doses, anti-RBD levels, and COVID-19 positivity through analysis of acute samples (N=2,576) and paired sample analysis (N=218). This thesis filled several important gaps in the literature on COVID-19 antibody responses as measured by RBD and NP antibody levels. The study found that having a breakthrough infection (as indicated by a positive SARS-CoV-2 PCR test at the acute visit among vaccinated individuals) was associated with a significantly lower level of anti-RBD antibody. This result was statistically-significant after controlling for key demographic factors as well as the time between the last vaccine and the acute visit, and the number of vaccine doses. Hence, this implies that vaccinated individuals with a more robust or persistent anti-RBD response are relatively protected from breakthrough infection compared to those individuals with a less robust response.

Acknowledgements

I would like to thank Dr. Brad Pearce and Dr. Eric Rogier for advising me on this project and helping us get to the core of this interesting dataset. I would also like to thank Dr. Brendan Flannery and Emma Noble for contributing data cleaning and analysis strategy efforts from the CDC flu team. I would like to thank Dr. Kumar for believing in me and inviting me to come work in his lab many years ago, which has been an incredible experience over the last two years. I would like to thank Ruchi Yadav and Ira Goldman for being true mentors and ambassadors of good science, showing me how to simplify my approach to research, while also pursuing excellence. I would also like to thank Maya Sands-Bliss fand Chad Robichaux for their R programming and problem-solving abilities.

Table of Contents

Background

Antibody levels in the blood can tell us a great deal about SARS-CoV-2 infections and how immune responses vary between different populations. Comparing different types of antibodies also further helps us differentiate between protection conferred by vaccination and/or protection from natural infection. Since the pandemic began in early 2020, clear differences have been identified in disease severity/vulnerability to infection; such factors include age, health condition and sex (CDC, 2020). Though serological data is a useful method of measuring COVID-19 prevalence, our ability to detect present and past infections is still evolving. The saturation of COVID-19 literature introduces additional variability and greater incidents of misclassification and selection bias. With variation between lab protocols and assays, researchers are working to standardize how we measure SARS-CoV-2 antibodies using WHO standard ratios and measurements. Changes in variant prevalence and repeated waves of infections also complicate scientific determinations as to what is really going on beneath the surface, especially when assays don't include variant specific targets.

The two main targets of the assay used for this study's purposes are the nucleocapsid protein (NP) and the receptor binding domain (RBD). Nucleocapsids are a structural protein of SARS-CoV-2 that forms complexes with genomic RNA. It plays a significant role in virus assembly. A receptor-binding domain (RBD) is a key part of the virus located on its 'spike' that allows it to dock to body receptors to gain entry into cells and lead to infection. Nucleocapsid and receptor binding domains help us differentiate whether someone has been vaccinated (only high levels of anti-RBD-antibodies are generated) or have had a natural infection (positive to anti-NP antibodies).

Examining total IgG antibody response to specific COVID-19 targets, and their increase after symptom onset, can help us predict how long antibodies will remain in the body. While there are many important factors we can assess as predictors of antibody response, defining antibody waning are crucial to this analysis. Understanding current patterns related to antibody responses helps us interpret our data

and understand what we expect to see as research questions that include temporality are explored. According to Assaid, it appears the majority of patients have detectable SARS-CoV-2 specific IgG antibodies after three months. (Assaid, Arich et al. 2023) Significant gaps remain in this study of symptomatic infections, however, as many patients present infection without fever or other symptoms. (Guan et al. 2020)

When examining the time it takes for an antibody level to rise and fall, NP antibodies have been seen to wane within months and decrease more quickly in young adults who are asymptomatic. Recent studies suggest IgG levels may also be higher in patients older than 50. (Assaid, Arich et al. 2023) We expect antibodies measured longitudinally to fall on a curve, where they rise and then fall again over 3-12 months. We expect to see N-protein specific response to be detectable in at least 90% of natural infections. (Pushpakumara 2023) Most PCR-confirmed infected persons are expected to be seropositive by two weeks after symptom onset (Okba, Muller et al. 2020) In some patients, seropositivity remained up to 12 months after symptom onset. (Chansaenroj, Yorsaeng et al. 2022) While NP levels rise and fall, patients who have had previous episodes of COVID-19 may have immunoglobulins that confer some level of protection. This relates to the clinical question of convalescent plasma treatment for COVID. (Bloch, Goel et al. 2023) Antibody levels/titers lead to many predictions regarding severity of infection and differing immune response between groups. In a retrospective analysis of 426 patients of convalescent collected from mild Covid-19 cases, individuals with high IgG antibody titers were observed having a longer stint of illness than those with lower levels. (Flieder 2023) According to Chansanrol, anti-S1 IgG and IgA titers "may stabilize following the infection period, while anti-N IgG levels increase immediately after SARS-CoV-2 infection but decline soon after, with a much shorter half-life" (Chansaenroj, Yorsaeng et al. 2022)). They also found that a relationship existed between disease severity and a stronger antibody-mediated immune response to SARS-CoV-2. (Chansaenroj, Yorsaeng et al. 2022)

Vaccines and Breakthrough infections

The tremendous health toll due to the COVID 19 pandemic spurred the unprecedented development of efficacious vaccines against symptomatic infection. In clinical trials, the initial mRNA-based vaccines achieved over 90% efficacy, meaning that those who receive the vaccine in initial clinical trials had better than 90% lower risk of developing disease than the group who received the placebo. However it is also important to consider real-world effectiveness, especially as the virus continues to mutate (Aleem et al, 2022). The current study focuses on real-world data in United States derived from the FLU VE Network. This thesis is focused on acute COVID-19 as documented within the overall FLU VE Network study.

It is generally believed that vaccine effectiveness varies between individuals based on a number of incompletely defined variables, including the duration between the vaccine and exposure to the virus (Tamandjou et al, 2023). Despite the effectiveness of vaccines, the occurrence of breakthrough infections, in which fully-vaccinated people become infected with SARS-CoV-2, underscores the importance of investigating the interplay between vaccination, immune responses, and symptomatic infection (Speiser et al, 2020). The aim of this thesis, using real-world antibody data, is to fill several crucial gaps in the association between vaccination and infection.

Vaccine dose is also an important difference to assess, with some studies finding the strongest antibody response after two doses, though protection differs depending on the variant, as seen with the rise of breakthrough infections during waves of the Delta variant. (CDC, 2022)

COVID-19 cases continue to spready with a wide breath of variants; Omicron infections, including XBB.1.5, BQ.1.1 and BQ1, account for the majority of infections. (CDC, 2022) According to Zheng, vaccinated children with Omicron breakthrough infection had 61.6 times higher IgG GMC than unvaccinated Omicron-infected children. Immune response seems to vary by age group, with children exhibiting different antibody responses, depending on the strain of infection. (Zheng 2022) Those with

lower antibody responses to Omicron may be at risk of reinfections. (Zheng). Reinfections can lead to higher risk of long covid symptoms. With such a diverse pool of variants, while Omicron continues to dominate the COVID burden in the US, age also appears to be an important factor to continue to explore. Age continues to be an important factor to consider when conducting serological studies of COVID-19. Children are as much as 5-9 times higher case rates than apparent with prevalence data after conducting serological studies. Some research suggests immune response among middle aged populations are more similar to that of younger individuals (Gallichotte 2022) Apart from age, antibody titers have been significantly associated with anosmia and ageusia, cough, and fever. (Huhn 2022). Spike protein antibody titers were higher in this study among those who presented with the above symptoms. Breakthrough infections among vaccinated individuals can be better understood by examining antibody titer changes. Without an understanding of asymptomatic SARS-CoV-2 prevalence, it becomes more difficult to assess the true effect specific factors like age and vaccine dosage have on an individual. Still, this makes serological data all the more important.

Last Immune Event and COVID Waves

Not knowing last immune event timing—asymptomatic cases, limits the inference we can make about the population included in the study. It will be difficult to attribute rising NP levels or RBD levels to only health characteristics or vaccine dose or event without knowing how recent their last infection may have been. We will use NP and date of last documented PCR confirmed infection from electronic records to assess how accurate our data appears. This sensitivity analysis will help assess how much noise vs. bias is added into the study through the unknown factor of asymptomatic cases and time since last infection for the majority of participants.

In this paper, we will examine two different aims related to both IgG response and vaccination status. Through our first aim, we can explore information related to the acute blood draw, with 2,765 participants analyzed after factoring in exclusion criteria.

Aim 1: Explore Relationship Between Vaccination and IgG response.

Comparing individuals who were vaccinated versus those who were not, we will determine whether the vaccinated subset have a higher IgG response at the time of acute infection than the unvaccinated individuals. By examining RBD and NP BAUs, we can analyze the linear relationship between antibody response and vaccination status. By considering the number of vaccine doses, it will be possible to further examine the role vaccination and level of vaccination play in immune response, when adjusting for other significant factors like age and sex. Out of the 2,804 participants in the study, 147 were given J&J for their first dose. Current literature suggests those who received J&J would have a lower antibody response but examining this relationship by number of doses will help reveal if that alters the overall results. Out of the 2,804 participants, only 213 covid positive individuals returned for a second blood draw, after exclusion for misclassification and inconclusive results/missing samples. Of those 213, we can further examine the same relationship between RBD and NP BAUs among vaccinated individuals. In our second aim, we will focus on the change in NP and RBD IgG antibody levels measured by BAU/ml between first and second blood draw.

Aim 2: Breakthrough Infections and Paired Analysis

Focusing on breakthrough infections (i.e., vaccinated subset only), we examined the relationship between pre-existing medical conditions, demographic variables, and specific vaccination characteristics on acute and convalescent IgG responses. We aim to explore if participant age, gender, race/ethnicity, date of illness onset, or self-reported chronic medical condition are associated with acute or convalescent BAUs (anti-NP and anti-RBD). We will also determine if vaccine type, vaccine dose, vaccine boosters, or time since last SARS-CoV-2 vaccine modifies this association (accounting for the time from self-reported symptom onset to the acute care visit and blood collection). While looking at just those who were infected and who are partially vaccinated, we can isolate our analysis to this group to see if there are any common attributes among this population. We will also look at symptoms and how those may or may not correlate with antibody changes within those who are vaccinated from the first to second blood draw. Health state is measured on a 1-5 likert scale from “poor” to “very good”. The relationship between this exposure and the binary outcome of infection vs. no infection will also be adjusted by age categories, age as a continuous variable, health care worker status, sex, and smoker status.

Manuscript

Introduction

Globally, as of October 19, 2022, there have been nearly 624 million confirmed cases of COVID-19, including over 6.5 million deaths, reported to the World Health Organization (WHO). COVID-19 cases continue to rise around the world, a global cause of mortality and debilitating long-term illness. While COVID-19 response has included large scale vaccination campaigns and heightened research into risk factors of severe disease, questions remain regarding how antibody responses differ among populations, and how they compare between naturally infected and vaccinated individuals. Examining total IgG antibody response to specific COVID-19 targets, and their increase over time, can lead to further inference regarding how infection and antibody protection may differ by health conditions, age group, vaccination status, time since infection, and other demographic characteristics. This can further inform preventive strategies and targeted protection for vulnerable populations.

Immunological Response

Apart from age, antibody titers have been significantly associated with anosmia and ageusia, cough, and fever. (Huhn 2022). Variation in symptoms especially within breakthrough infections among vaccinated individuals can be better understood by examining antibody titer changes.

Targets

The two main targets of the assay used for this study's purposes are the nucleocapsid protein (NP) and the receptor binding domain (RBD). Nucleocapsids are a structural protein of SARS-CoV-2 that forms complexes with genomic RNA. It plays a significant role in virus assembly. A receptor-binding domain (RBD) is a key part of the virus located on its 'spike' that allows it to dock to body receptors to gain entry into cells and lead to infection. Nucleocapsid and receptor binding domains help us

differentiate whether someone has been vaccinated (only high levels of anti-RBD-antibodies are generated) or have had a natural infection (positive to anti-NP antibodies).

Methods

Exclusion Criteria

Of the 2,804 participants in the study, several were excluded due to mistyping of dates or missing sample information (figure 1). This data is cross-sectional and was collected from seven sentinel sites through the Flu VE network. Patients presenting with COVID-19 symptoms at select clinics were considered for study inclusion. For paired analysis, only PCR lab-confirmed SARS-CoV-2 infected individuals were invited to return.

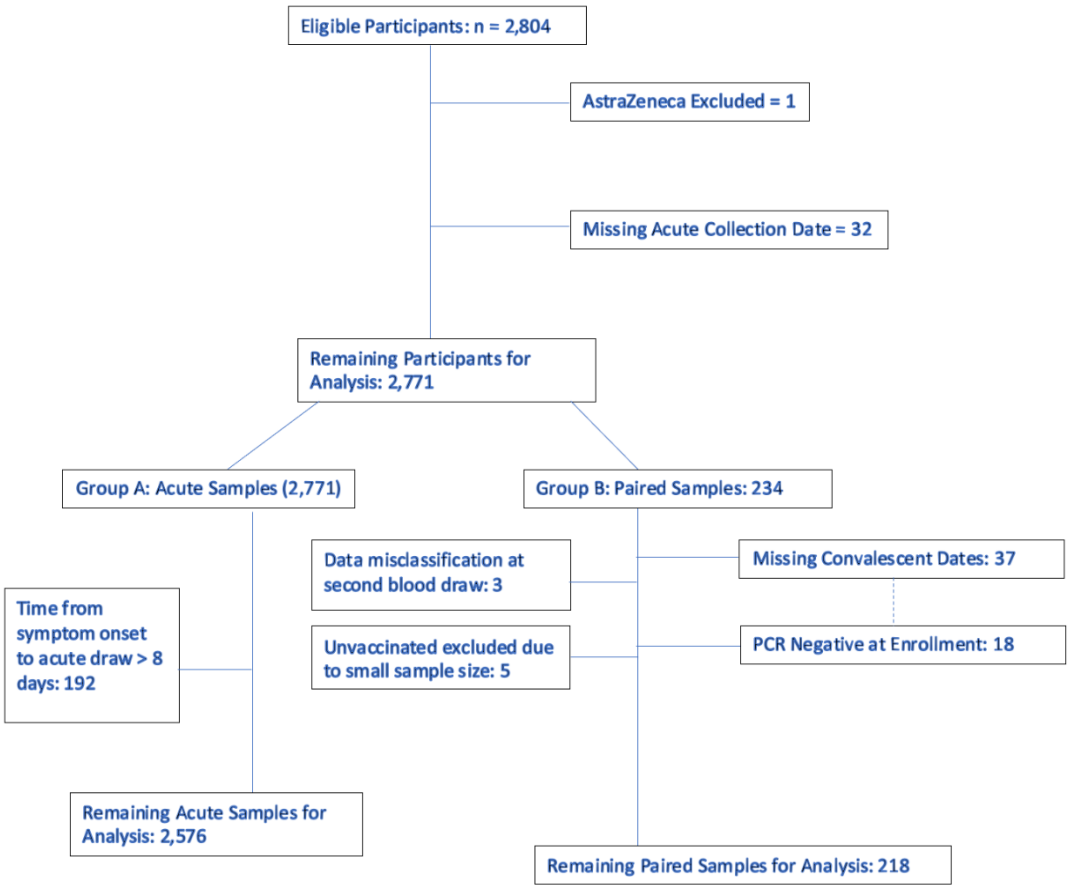

Figure 1 *Exclusion Criteria (N = 2,804)*

Site-Specific Cross-Sectional Sampling

Characteristics of the sample.

Timing of pandemic waves is also an important limiting factor, with the use of cross-sectional data. With participants showing up at clinics with symptoms, this serves as a convenience sample spread over seven different sites (figure 2). Within those sites are also different sample sizes (table 1). Our external

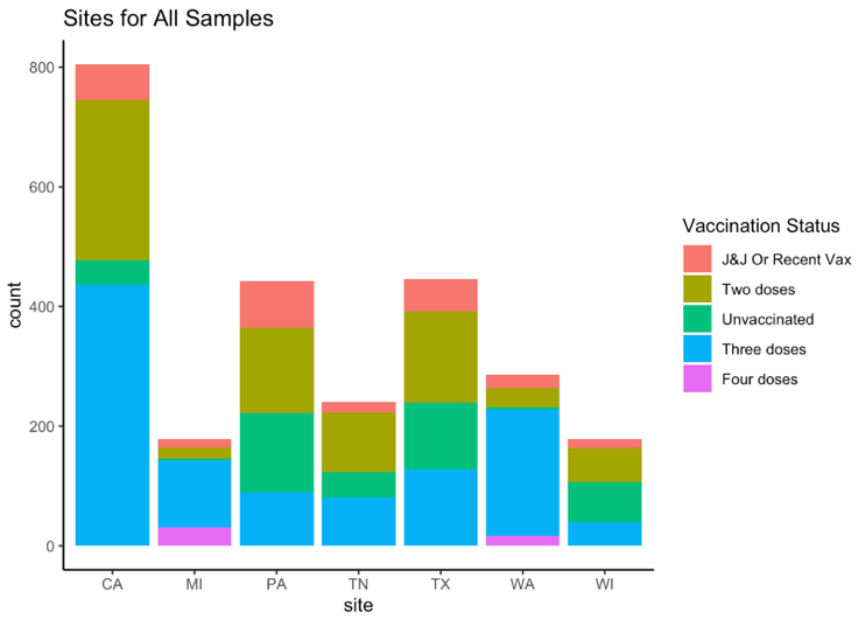

Figure 2 *Sites by Vaccination Status (Source: Flu VE Network)*

	Overall (N=2576)
site	
CA	805 (31.3%)
MI	178 (6.9%)
PA	443 (17.2%)
TN	240 (9.3%)
TX	446 (17.3%)
WA	286 (11.1%)
WI	178 (6.9%)

Table 1 *Overall site sampling*

greatly limited due to the method of collection and sampling strategy, although we can adjust for some of these factors when examining seroprevalence. We still are analyzing samples across areas of different COVID-19 prevalence over a year span, compared across different timeframes. California accounted for 31.3% of the samples processed with Pennsylvania and Texas both also including a majority of the samples with 17.2% and 17.3% respectively.

There is clear variation in the number of samples received from sites. It is important to note J&J vaccinated individuals were separated from mRNA vaccine categorization for dosage, as well as those who had a recent vaccination dose (Table 2). J&J or Recent Vax includes individuals who were administered a second dose within 14 days of sample collection, or any booster dose within 7 days of sample collection. They were excluded due to biological reasons, since they didn't have enough time for full protection from their most recent dose.

Of the 2,576 participants included, 32.1% were PCR positive for COVID-19 at enrollment.

	Unvaccinated (N=404)	J&J Or Recent Vax (N=262)	Two doses (N=768)	Three doses (N=1095)	Four doses (N=47)	Overall (N=2576)
Lab confirmed PCR Infection						
negative	276 (68.3%)	183 (69.8%)	545 (71.0%)	714 (65.2%)	31 (66.0%)	1749 (67.9%)
positive	128 (31.7%)	79 (30.2%)	223 (29.0%)	381 (34.8%)	16 (34.0%)	827 (32.1%)

Table 2 *Lab confirmed COVID-19 cases at first blood draw stratified by dose*

Patients were tested in the clinic with PCR when they entered with symptoms, and a second test was also sent for lab testing. If one of these two tests, or both, were positive, they were classified as having a current PCR confirmed COVID-19 infection. Below, we stratify descriptive statistics based on those that tested positive and those that tested negative upon enrollment.

Recruitment and Blood Sampling

Blood samples for this study were collected at the time of an acute clinical visit for COVID-19 symptoms, and additional information on medical history, including the onset of illness, was likewise collected. The selection of groups for analysis is shown in figure 1. As elaborated below, this thesis focuses on antibody binding levels to the SARS-CoV-2 protein antigens, the nucleocapsid protein (NP) and the receptor binding domain (RBD). The results expressed are the standardize data on a linear scale

by analyzing the Median Fluorescent Intensity scores converted to Binding Antibody Units (BAU). For simplicity's sake, this quantitative measure of standardized antibody binding to specific antigens is referred to (e.g in some tables) as "RBD" or "NP", which represents anti-RBD BAU binding level or the ani-NP BAU binding level, respectively, unless otherwise specified.

Lab Analysis of Blood Spots for Quantitative Assessment of Antibody Level (BAU/ml)

The blood spots were then run on a 7plex assay at the CDC laboratory. This assay utilizes magnetic microspheres coupled with unique recombinant proteins specific for SARS-CoV-2 (RBD, NP). The assay also includes four different internal controls for monitoring each step of assay performance. External positive control, negative control, and calibrator reagents were also provided in the kit and were run in duplicate on every assay plate. Extracted samples from dry blood spots, tested in duplicate, were diluted 1:400 in assay kit sample dilution buffer, immediately mixed with the antigen-coated microspheres in a 96-well plate and incubated for 20 minutes by gentle shaking at room temperature protected from light. Plates were washed four times with assay wash buffer, and serum IgG antibodies were detected using anti-human IgG conjugated to phycoerythrin by incubation at 20 min under gentle shaking protected from light. Plates were then washed four times and the microspheres resuspended in wash buffer and analyzed using a Luminex MAGPIX instrument and a Luminex LX 200 flow analyzer (Luminex Corporation, Austin, TX) with a target of 50 beads per region. While my thesis mainly concerns the antibody binding as a continuous measure, we will also have data on the assay cutoff, which is established by the manufacturer. This was used for a scoring test of positive samples and any indeterminate samples could be repeated as recommended by the test manufacturer.

RESULTS

Descriptive Statistics for Overall-Sample-Set

As shown in table 3, median NP BAU level at acute onset among COVID-confirmed patients was 4.18, while those who were negative for COVID had a median NP BAU level of 7.49. Of the positive cases, 50.2% were under the age of 40, while 40.5% were between 40 and 65. The majority of participants

Overall Descriptive Statistics (By PCR lab confirmed infection)

	Total (N=2576)	negative (N=1749)	positive (N=827)
RBD at Baseline (BAU/ml)			
Mean (SD)	2120 (1790)	2220 (1820)	1930 (1730)
Median [Min, Max]	1800 [0.351, 8400]	1990 [0.351, 8400]	1460 [0.423, 7650]
NP at Baseline (BAU/ml)			
Mean (SD)	84.0 (281)	101 (305)	48.0 (220)
Median [Min, Max]	6.18 [0.103, 3560]	7.49 [0.103, 3560]	4.18 [0.151, 3240]
Age Group (Years)			
0-40	1366 (53.0%)	951 (54.4%)	415 (50.2%)
40-65	961 (37.3%)	626 (35.8%)	335 (40.5%)
> 65	249 (9.7%)	172 (9.8%)	77 (9.3%)
Sex			
Female	1631 (63.3%)	1115 (63.8%)	516 (62.4%)
Male	942 (36.6%)	632 (36.1%)	310 (37.5%)
Missing	3 (0.1%)	2 (0.1%)	1 (0.1%)
Race			
White	1593 (61.8%)	1114 (63.7%)	479 (57.9%)
Black	121 (4.7%)	81 (4.6%)	40 (4.8%)
Asian	222 (8.6%)	128 (7.3%)	94 (11.4%)
Other	100 (3.9%)	65 (3.7%)	35 (4.2%)
Hispanic	508 (19.7%)	346 (19.8%)	162 (19.6%)
Missing	32 (1.2%)	15 (0.9%)	17 (2.1%)
Days Between Symptom Onset and Enrollment			
Mean (SD)	3.01 (1.81)	3.14 (1.81)	2.72 (1.78)
Median [Min, Max]	3.00 [-6.00, 7.00]	3.00 [-1.00, 7.00]	2.00 [-6.00, 7.00]
Time Since Last Known COVID Infection (Days)			
Mean (SD)	253 (199)	276 (171)	206 (241)
Median [Min, Max]	205 [1.00, 759]	283 [21.0, 702]	28.0 [1.00, 759]
Missing	2237 (86.8%)	1521 (87.0%)	716 (86.6%)
Evidence of Previous Infection (BAU/ml Cutoff)			
Negative	1351 (52.4%)	833 (47.6%)	518 (62.6%)
Positive	1225 (47.6%)	916 (52.4%)	309 (37.4%)

Source: Flue VE Network

Table 3 *Descriptive Statistics for acute blood draw/baseline of overall sample set*

were female (63.3%). Of those COVID positive[1], 37.4% showed evidence of a positive infection in the past (calculated by NP seropositivity cutoff explained below).

Vaccination Status and PCR Test Results for COVID-19

As shown in figure 3, the majority of participants (1,886) tested negative for PCR covid tests at acute blood draw. Still, 827 tested positive.

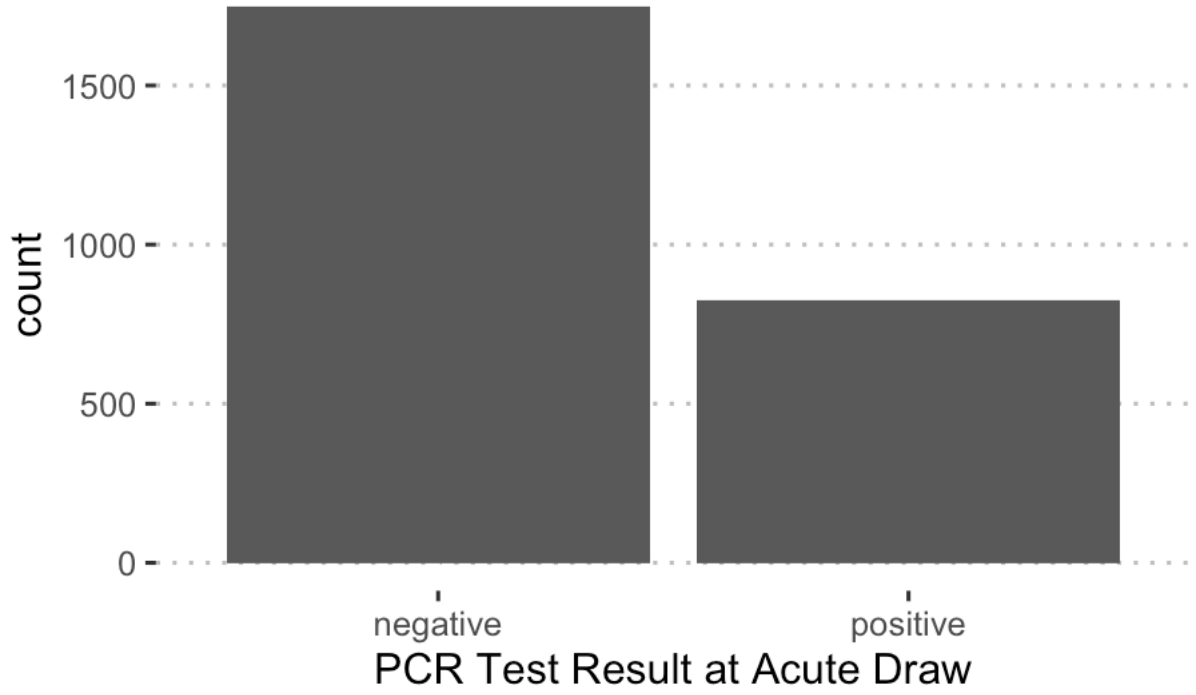

Figure 3 *Participants who tested positive at enrollment with lab confirmed PCR (either in lab or clinic)*

Age and Race

[1] *Patients were tested in the clinic with PCR when they entered with symptoms, and a second test was also sent for lab testing. If one of these two tests, or both, were positive, they were classified as having a current PCR confirmed COVID-19 infection. Below, we stratify descriptive statistics based on those that tested positive and those that tested negative upon enrollment.*

There was an approximately normal distribution of age among the study participants with a median age of 41. The majority of participants were white, followed by Hispanic and Asian/non-Hispanic participants (figure 4). The histogram below (figure 5) shows the distribution of age for participants in the study (N=2,768). Figure 8 shows this breakdown by race.

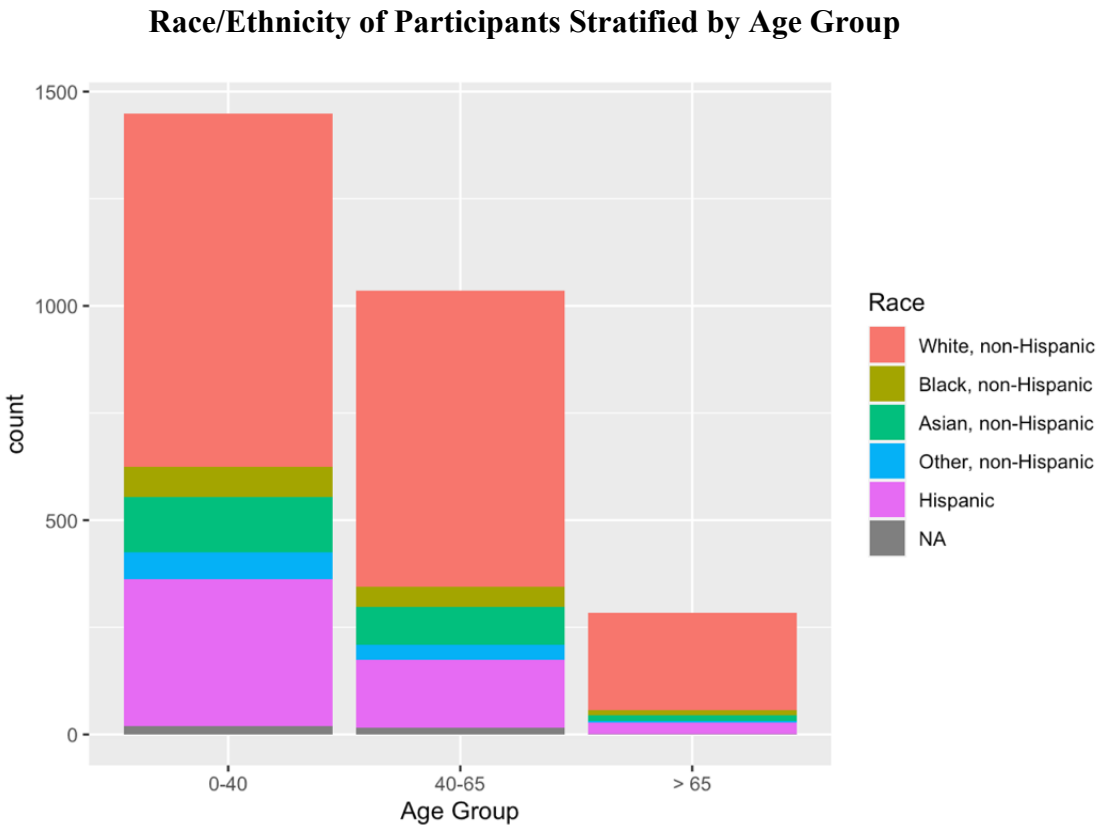

Figure 4 *Race/ethnicity of patients with age group stratification*

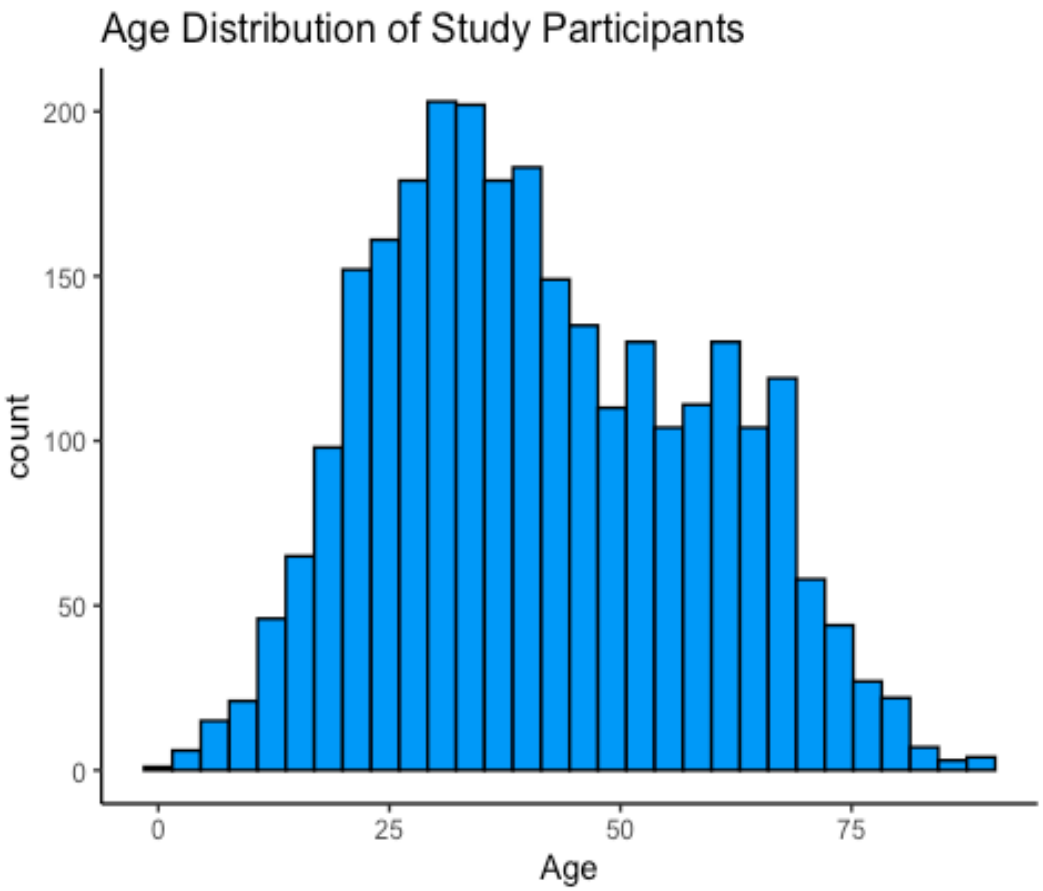

Figure 5 *Age of Study participants (normally distributed)*

Exclusion of 8+ Days Since Symptom Onset

Each participant recorded how the number of days from symptom onset to their blood draw. As shown here, we can see in figure 6 that NP natural infection antibodies appear to rise if participants arrived later than 10 days after the start of their symptoms. This suggests some acute NP levels are related to current infection and not evidence of previous infection.

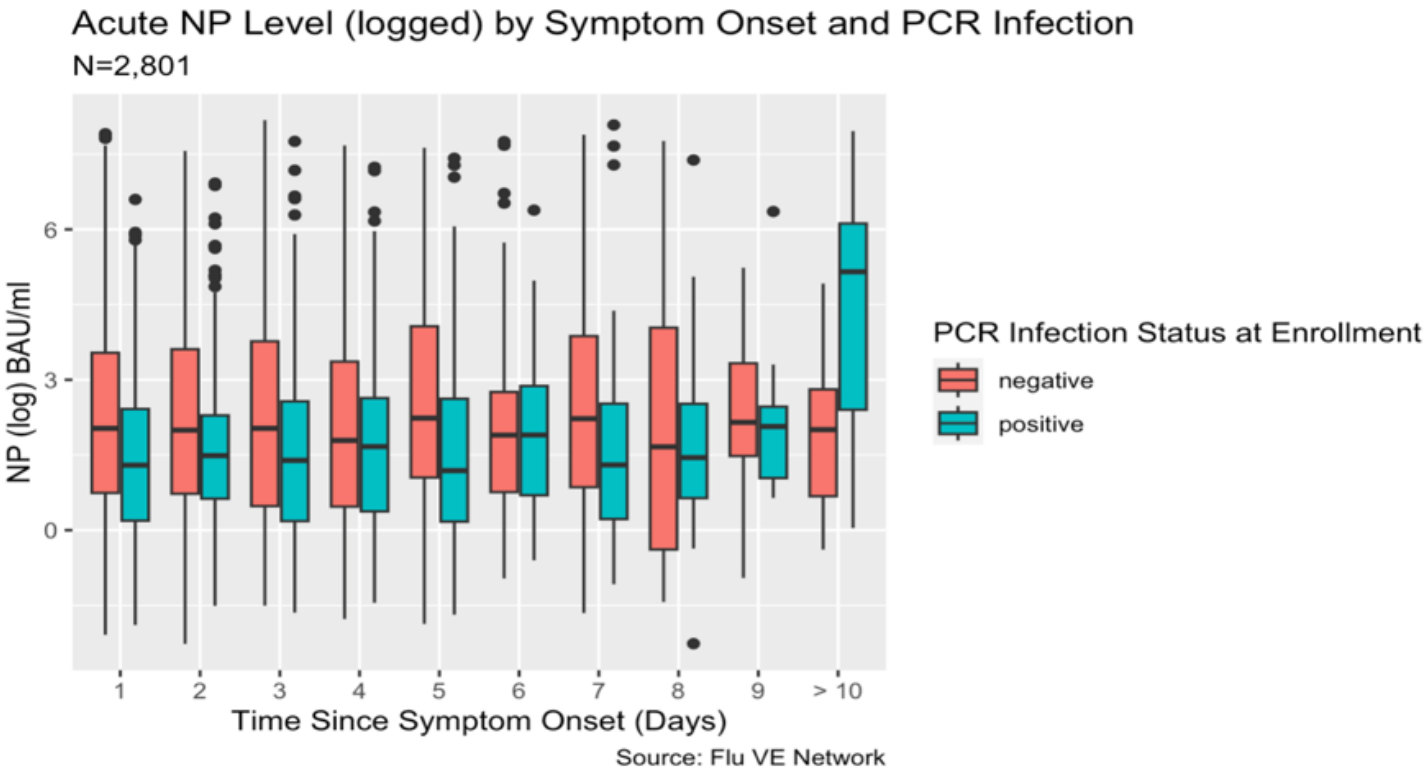

Figure 6 *Patients at 8+ Days start showing antibodies from current infection at acute blood draw (NP logged)*

Group A Study: RBD and NP Distributions

We anticipated that all vaccinated individuals (with at least one dose) would have detectable IgG antibodies to RBD at the baseline visit, since this is the target of the vaccines. Further, we anticipated that detection of NP antibodies at baseline is an indicator of prior infection (a SARS-CoV-2 infection that occurred before the index infection and blood collection). Fig 7a shows the distribution of BAU values at baseline for RBD and figure 7b shows the log transformed values of that same RBD baseline data. Fig 7c shows the NP distribution at baselines, and figure 7d shows the same data log transformed. NP outliers greatly skewed the distribution. This may be due to recent COVID-19 infection or a large number of days between symptom onset and the day the patient entered the clinic for enrollment.

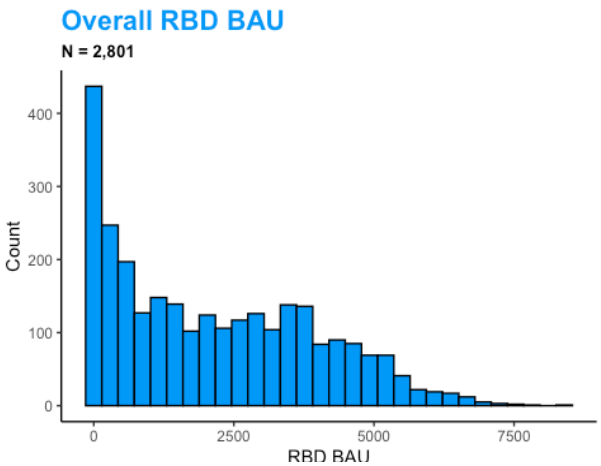

Figure 7a *RBD distribution (BAU/ml) at baseline*

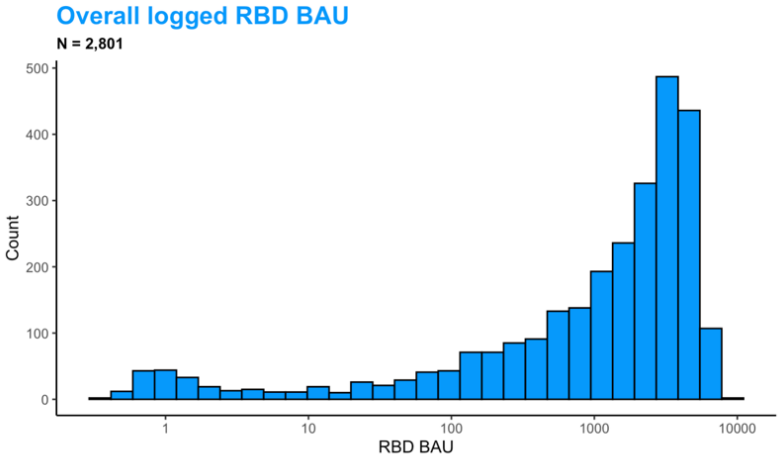

Figure 7b *RBD distribution (BAU/ml) at baseline* ***(log)***

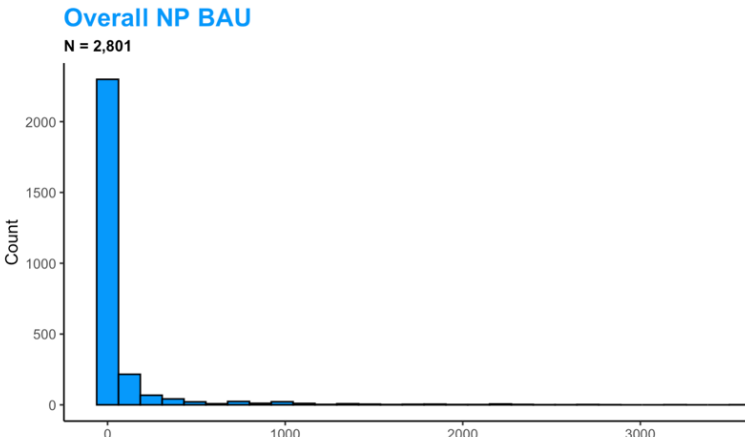

Figure 7c *NP distribution (BAU/ml) at baseline*

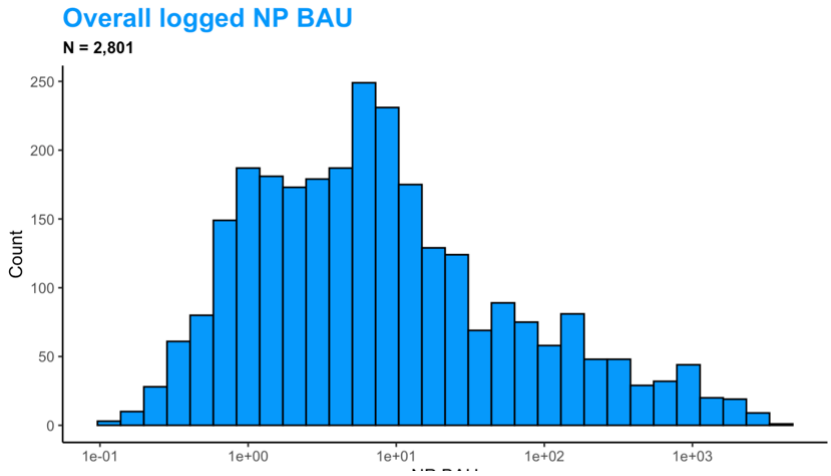

Figure 7d *NP distribution (BAU/ml) at baseline* ***(log)***

Aim 1a: Vaccination and Antibody Titers

When examining the baseline collection, we hypothesized that vaccinated individuals will have higher BAU for RBD levels (a stronger immune response). Here, RBD and NP at acute blood draw serves as our outcome and vaccination status/dose serves as our main predictor (table 4).

Model 1: Linear Regression with Outcome: RBD at Baseline

RBD (BAU/ml)

$$Y(RBD) = vaccinedosex_1 + timesincelastdosex_2 + vaccinetypex_3 + agex_4 + racex_5 + e$$

RBD at Baseline (BAU/ml) and Vaccination Status

	RBD at Baseline(BAU/ml)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	1122.35	490.81 – 1753.90	**0.001**
Vaccination Status: Two doses	923.01	306.35 – 1539.66	**0.003**
Vaccination Status: Three doses	2371.79	1752.80 – 2990.78	**<0.001**
Vaccination Status: Four doses	3306.46	2537.97 – 4074.96	**<0.001**
p covmanu 1: PFIZER	-325.46	-475.12 – -175.80	**<0.001**
Age(Years)	-7.38	-11.95 – -2.80	**0.002**
Sex: Male	17.23	-132.69 – 167.16	0.822
Race: Black	-184.70	-553.60 – 184.21	0.326
Race: Asian	-240.95	-484.88 – 2.97	0.053
Race: Other	75.19	-287.89 – 438.28	0.685
Race: Hispanic	84.90	-100.79 – 270.58	0.370
Days Between Symptom Onset and Enrollment	9.44	-30.68 – 49.56	0.644
Observations	1917		
R^2 / R^2 adjusted	0.185 / 0.180		

***Table 4**: model 1: RBD at baseline compared to vaccine doses (fully vaccinated levels)*

Plot 1: RBD and Vaccination Status

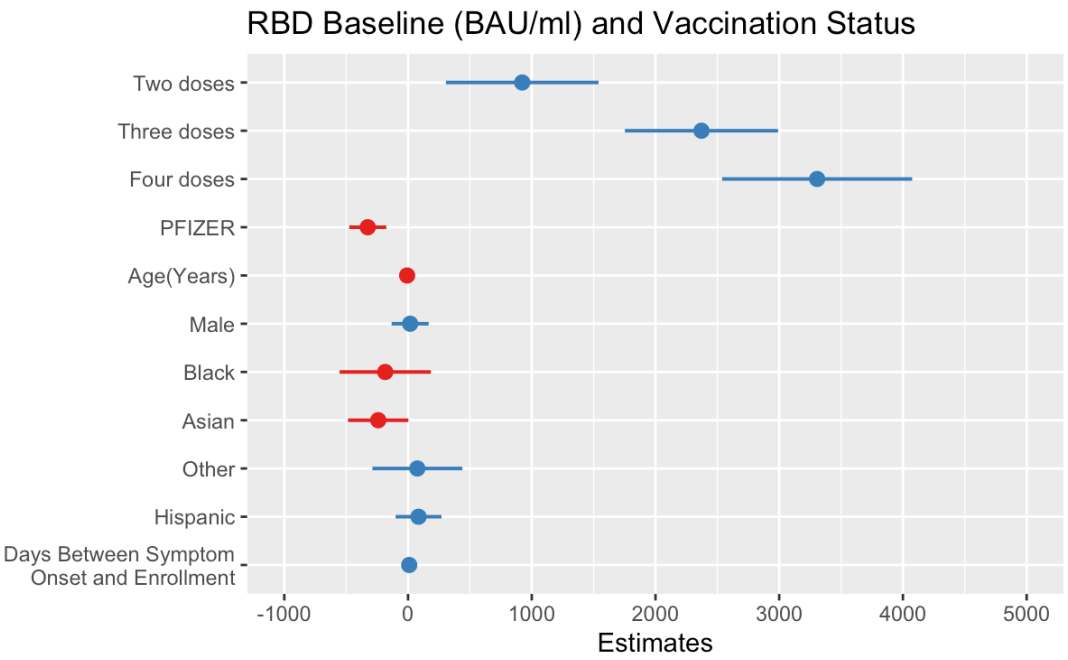

Figure 8 *plot of model 1: RBD and vaccine status controlling for demographics*

Interpretation of Model 1

As expected, four doses, with a point estimate of 3306.46 (2537.97, 4074.96), has the greatest effect on RBD level at baseline (figure 8), followed by three doses (2371.79 [1752.80, 2990.78] and two doses (923.01 [306.35, 1539.66], controlling for demographics and days between symptom onset and enrollment. The median of RBD values at blood draw was 1847.481. For every ten-year change in age, the expected value of RBD BAU decreases on average by approximately 73.8 (-119.5,-28) BAU/ml, controlling for vaccination status, time since last dose, vaccine type, sex, race and days between symptom onset and enrollment. For those with PFIZER for their first dose, there appears to be -325.46 RBD (-475.12, -175.8) BAU/ml difference than those who had Moderna for their first dose. When adjusting for time since last vaccine dose, the model remains the same in positive and negative effect estimates (figure 9).

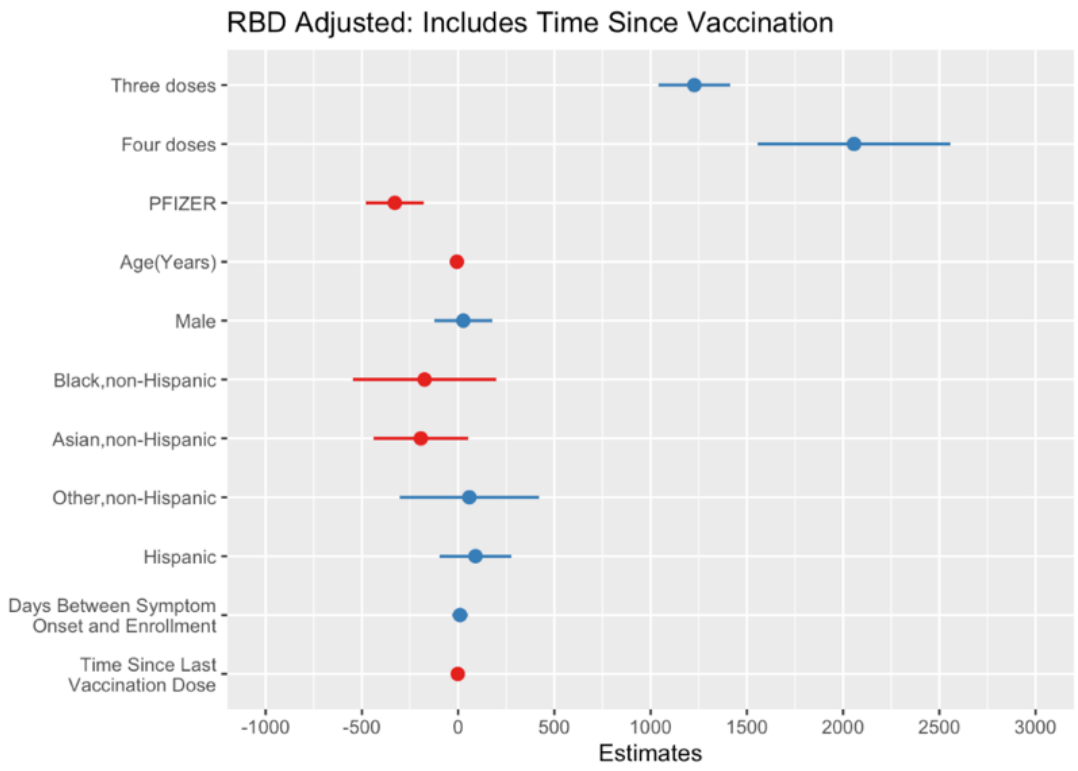

Figure 9 *Adjusted model that includes time since vaccination when examining RBD and Vaccination Status*

Sensitivity Analysis:

Known prior infection and Seropositivity (NP BAU/ml Cutoff of 6.9). This is calculated from the MFI ratio cutoff of 1.2 from the Tetracore validated assay. There were 365 people who had reported previous infections based on electronic health record data (table 5). Of those 365, when filtering to only these HER confirmed prior symptomatic infections, 74.2% tested positive for prior infection when examining mfi and BAU cutoffs. It is important to note, however, that 211 of those individuals tested positive by PCR more than 90 days prior, meaning NP levels would have been expected to wane considerably.

	Overall (N=300)
Evidence of Previous Infection (BAU/ml Cutoff)	
Negative	68 (22.7%)
Positive	232 (77.3%)
Previous EHR Confirmed Infection by Time Category (Days)	
<30	53 (17.7%)
30-90	36 (12.0%)
> 90	211 (70.3%)

***Table 5**: previous EHR confirmed infections and seropositivity*

Model 2: Unvaccinated Sub-Population: RBD at Acute Draw and PCR-Confirmed Infection

We also examined whether RBD BAU level at acute draw is associated with likelihood of infection at enrollment. We hypothesize that those individuals who had the acute blood draw but tested negative for SARS-COV-2 by PCR, would have higher levels of RBD BAU, either because of prior infection or prior vaccination, or both (table 6). The logistic regression for this model is included below

Analyzing Subset of ONLY Unvaccinated Individuals

	Lab confirmed PCR Infection		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	0.16	0.08 – 0.30	**<0.001**
RBD at Baseline(BAU/ml)	1.00	1.00 – 1.00	0.068
Sex: Male	1.12	0.71 – 1.77	0.615
Race: Black	1.00	0.39 – 2.35	0.995
Race: Asian	4.69	1.14 – 20.82	**0.032**
Race: Other	1.83	0.53 – 5.84	0.313
Race: Hispanic	1.35	0.72 – 2.48	0.338
Age(Years)	1.03	1.02 – 1.05	**<0.001**
Observations	381		
R^2 Tjur	0.063		

***Table 6**: Model 2: COVID-19 positivity and RBD at Baseline*

with the outcome variable of covid positive vs. negative test and the population subset only those who are not vaccinated. The relationship of RBD to vaccinated individuals is considered further in aim 1b.[2]

Interpretation of Model 2

There was no clear relationship that appeared between RBD level and COVID positive result among unvaccinated individuals when controlling for age, sex and race (figure 10). The odds of testing positive for COVID-19 for Asian participants were 4.69 (1.14, 20.82) times the odds of the odds of testing

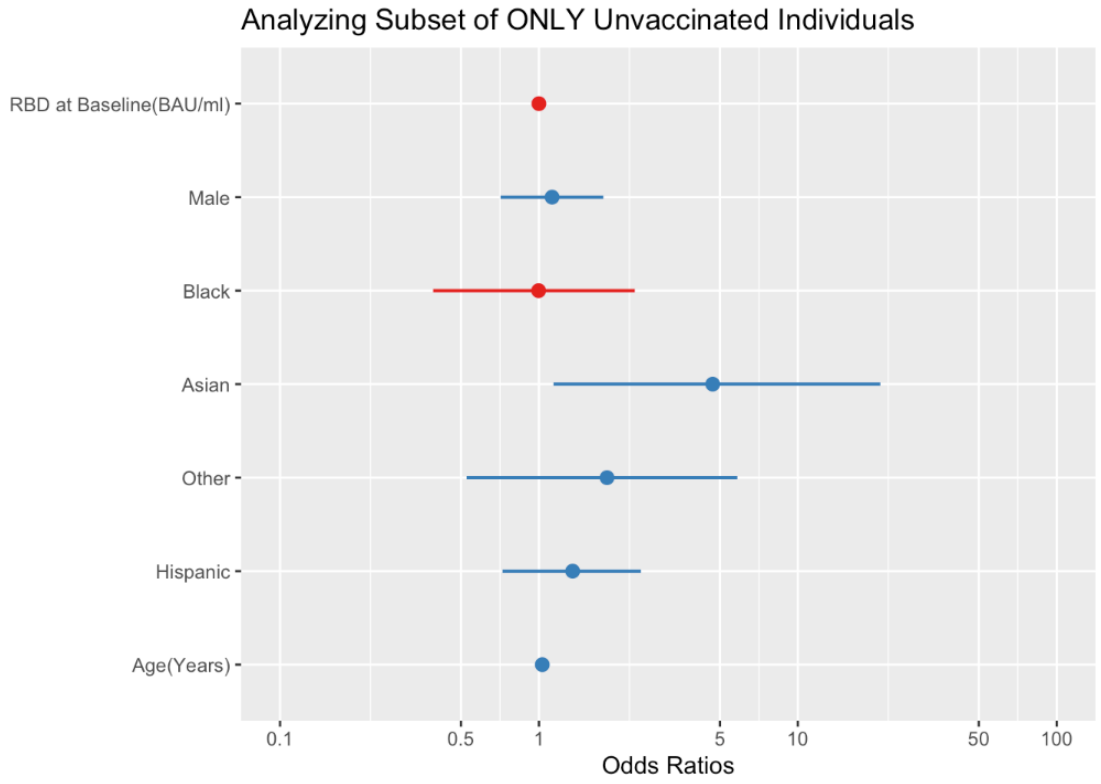

Figure 10 *Plot of unvaccinated individuals and point estimates for model 2 regression by covid positivity (PCR Confirmed)*

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[2] **Referent group for race:** white

Age: (Years)

RBD*at_least_one_dose: referent group is unvaccinated

positive for COVID-19 for white participants among unvaccinated individuals when controlling for RBD baseline, age, sex and race.

Aim1b: Vaccine Only Comparison & Breakthrough Infections

Model 3[3]: Breakthrough Infections: RBD level compared to PCR positivity among Vaccinated Individuals (table 7)

Here are the results for our vaccine only population (N = 2,285):

$y = vaccinedosex_1 + timesincelastdosex_2 + vaccinetypex_3 + agex_4 + racex_5 + e$

With Time Since Last Vaccination

	RBD at Baseline(BAU/ml)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	2255.06	1950.72 – 2559.41	**<0.001**
PCR Confirmed Infection at First Blood Draw: positive	-338.40	-494.01 – -182.79	**<0.001**
Vaccination Status: Three doses	1272.23	1085.03 – 1459.43	**<0.001**
Vaccination Status: Four doses	2072.47	1571.90 – 2573.04	**<0.001**
Race: Black	-187.94	-559.97 – 184.08	0.322
Race: Asian	-146.52	-392.63 – 99.59	0.243
Race: Other	71.02	-290.89 – 432.93	0.700
Race: Hispanic	118.71	-67.28 – 304.71	0.211
Sex: Male	48.54	-101.93 – 199.00	0.527
Age(Years)	-5.27	-9.85 – -0.70	**0.024**
Time Since Last Vaccination Dose	-1.65	-2.57 – -0.73	**<0.001**

***Table 7**: Model 3 Linear regression RBD at baseline compared to PCR positivity at first blood draw*

[3] **vaccine dose referent group:** two doses (since unvaccinated are not included and we are using a fully vaccinated variable that does not include "at lease one dose" criteria)

vaccine type referent group: Moderna

race referent group: white

Interpretation of Model 3

Among vaccinated individuals, those who were COVID-19 positive on average had a -338.40 (-494.01, -182.79) RBD BAU/ml level at baseline compared to those who were PCR negative and vaccinated (<0.001) (figure 11). Three doses (1272.23 [1085.03, 1459.43], and four dose (2072.47 [1571.90, 2573.04] vaccine levels compared to two doses were all associated with higher RBD levels at baseline. For every 10-year increase in age, RBD at Baseline, on average, was 52.7 (-98.5, -7.0) RBD BAU/ml lower among vaccinated individuals, controlling for age, sex race, and time since last vaccination dose. On average, for every one day increase between time since last vaccination dose, RBD at baseline was -1.65 lower (-2.57, -0.73) when controlling for vaccine status, race, age and sex.

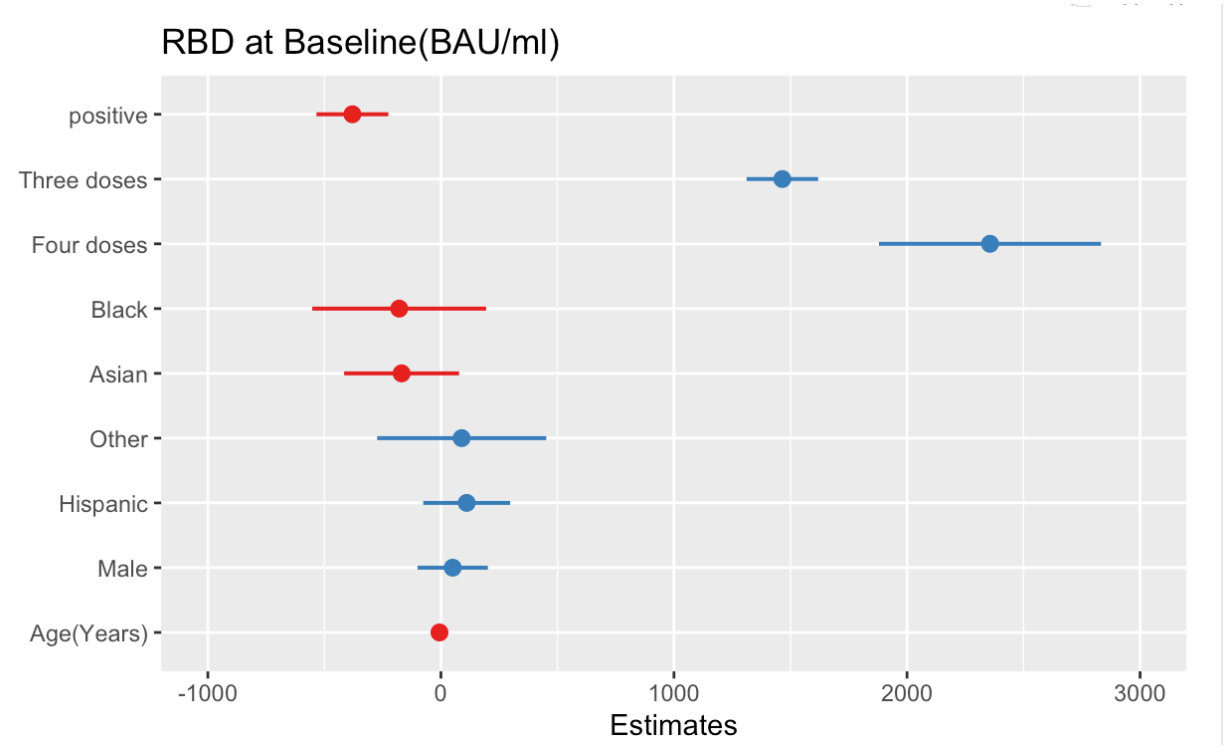


Figure 11 *Plot for Model 3: RBD at Baseline compared to PCR result at baseline among vaccinated individuals*

Group B (Paired) Analysis: Aim 2

Of those who were COVID-19 positive through lab-confirmed PCR at enrollment, all were invited back for a second blood draw. As shown in the exclusion criteria above, 198 total who returned and met inclusion standards. Once the data were cleaned, there were only five individuals in the paired data who were unvaccinated. Therefore, this analysis became a breakthrough infection only analysis. All paired samples were included based on the criteria of having a PCR positive test at enrollment. We are excluding unvaccinated from the analysis.

Descriptive Statistics for Paired Sub-Set

Time between visits varied for paired sampling (figure 12). As shown below, we have divided time between visits into categories of days, with some significant outliers present in the 21-65 days category. These time categories are based on what we expect to see biologically with infections: an increase in NP antibodies until approximately 6-8 weeks, followed by a slow waning at that point.

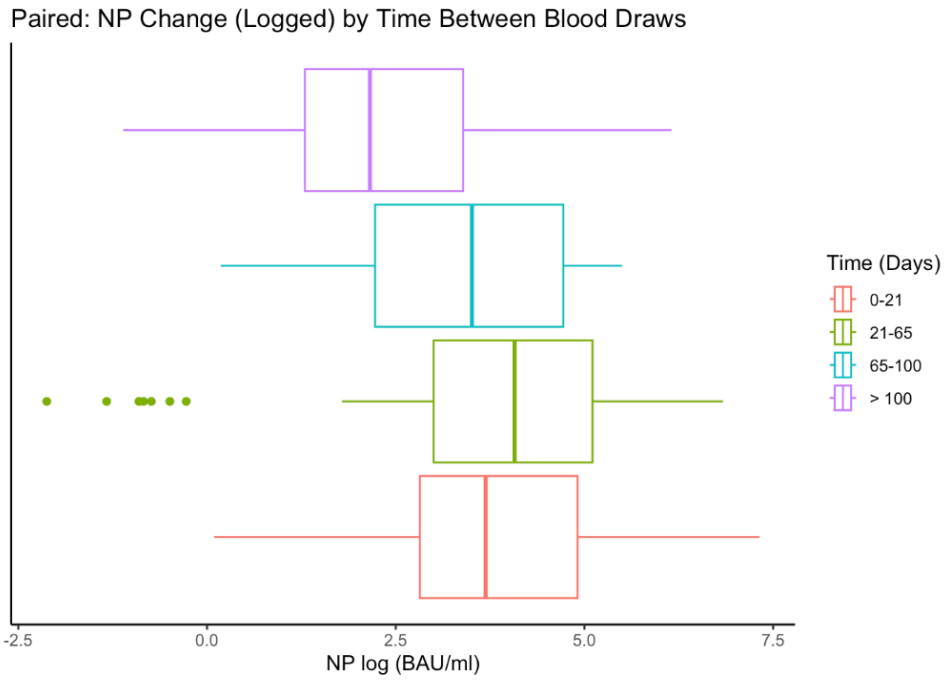


Figure 12 *NP change between the two blood draws logged for scale and categorized by time between blood draws*

Descriptive Statistics for Paired Samples

	J&J Or Recent Vax (N=11)	Two doses (N=26)	Three doses (N=154)	Four doses (N=8)	Overall (N=199)
NP at Baseline (BAU/ml)					
Mean (SD)	51.1 (133)	163 (644)	34.0 (187)	13.7 (14.3)	51.0 (287)
Median [Min, Max]	4.57 [0.793, 451]	2.54 [0.359, 3240]	5.02 [0.368, 2130]	8.02 [2.76, 44.1]	4.85 [0.359, 3240
RBD at Baseline (BAU/ml)					
Mean (SD)	1660 (1670)	1340 (1620)	2500 (1410)	3580 (860)	2350 (1510)
Median [Min, Max]	1300 [113, 5140]	524 [5.74, 5120]	2520 [1.17, 6140]	3510 [2290, 4910]	2290 [1.17, 6140
Health Condition (no/yes)					
No	7 (63.6%)	16 (61.5%)	111 (72.1%)	3 (37.5%)	137 (68.8%)
Yes	4 (36.4%)	9 (34.6%)	33 (21.4%)	2 (25.0%)	48 (24.1%)
Missing	0 (0%)	1 (3.8%)	10 (6.5%)	3 (37.5%)	14 (7.0%)
Age Group (Years)					
0-40	3 (27.3%)	14 (53.8%)	63 (40.9%)	1 (12.5%)	81 (40.7%)
40-65	8 (72.7%)	10 (38.5%)	65 (42.2%)	3 (37.5%)	86 (43.2%)
> 65	0 (0%)	2 (7.7%)	26 (16.9%)	4 (50.0%)	32 (16.1%)
Race					
White	5 (45.5%)	14 (53.8%)	92 (59.7%)	4 (50.0%)	115 (57.8%)
Black	1 (9.1%)	0 (0%)	3 (1.9%)	0 (0%)	4 (2.0%)
Asian	1 (9.1%)	4 (15.4%)	23 (14.9%)	4 (50.0%)	32 (16.1%)
Other	0 (0%)	3 (11.5%)	10 (6.5%)	0 (0%)	13 (6.5%)
Hispanic	3 (27.3%)	5 (19.2%)	25 (16.2%)	0 (0%)	33 (16.6%)
Missing	1 (9.1%)	0 (0%)	1 (0.6%)	0 (0%)	2 (1.0%)
Sex					
Female	7 (63.6%)	15 (57.7%)	83 (53.9%)	6 (75.0%)	111 (55.8%)
Male	4 (36.4%)	11 (42.3%)	71 (46.1%)	2 (25.0%)	88 (44.2%)

Table 8: *Descriptive statistics for paired subset of individuals for paired analysis*

For the paired samples, we have included the descriptive data in table 8. Important to note that only five of the paired samples were unvaccinated, so we excluded them altogether. Age was still normally distributed (figure 13) and the majority of participants in the paired subset were white (figure 14).

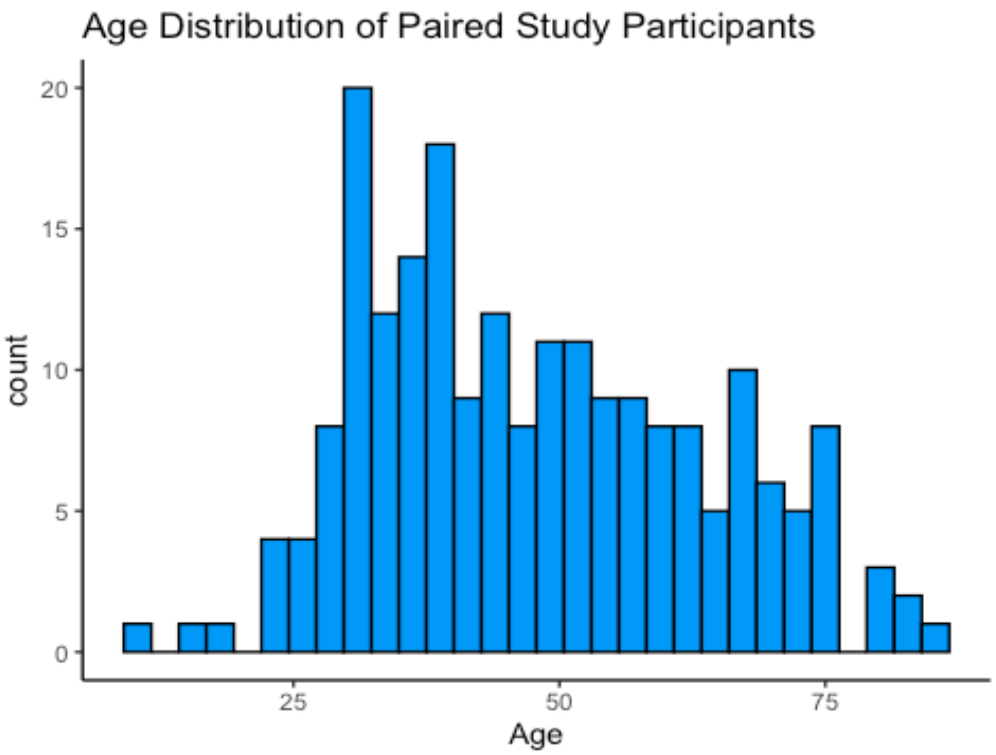


Figure 13 *Distribution of Age among participants*

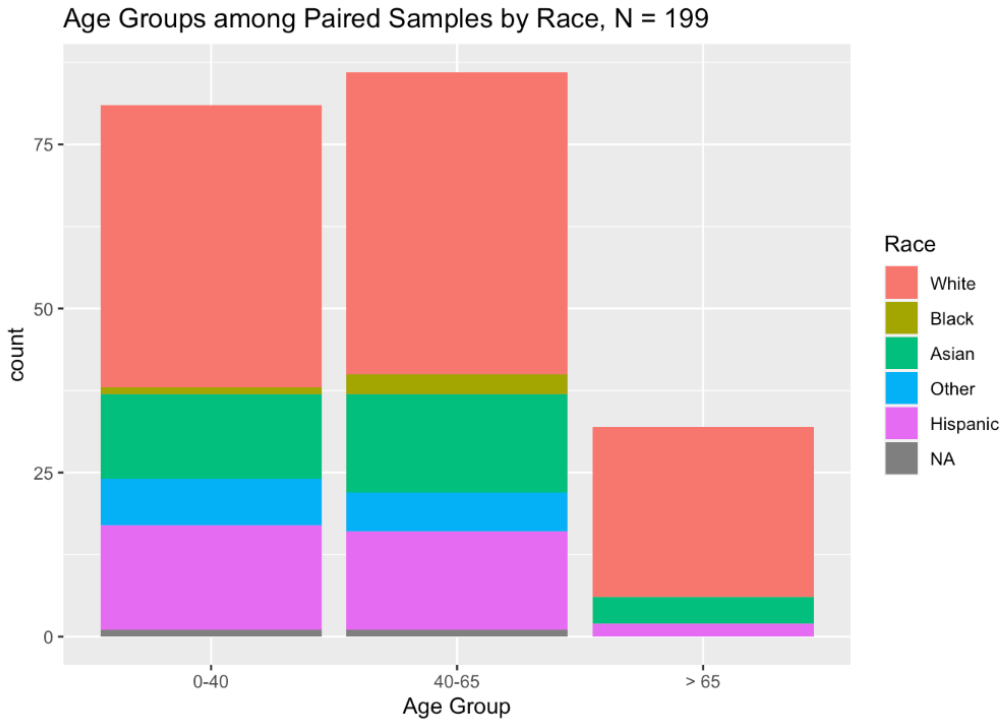


Figure 14 *Race/ethnicity distribution by age group among paired samples*

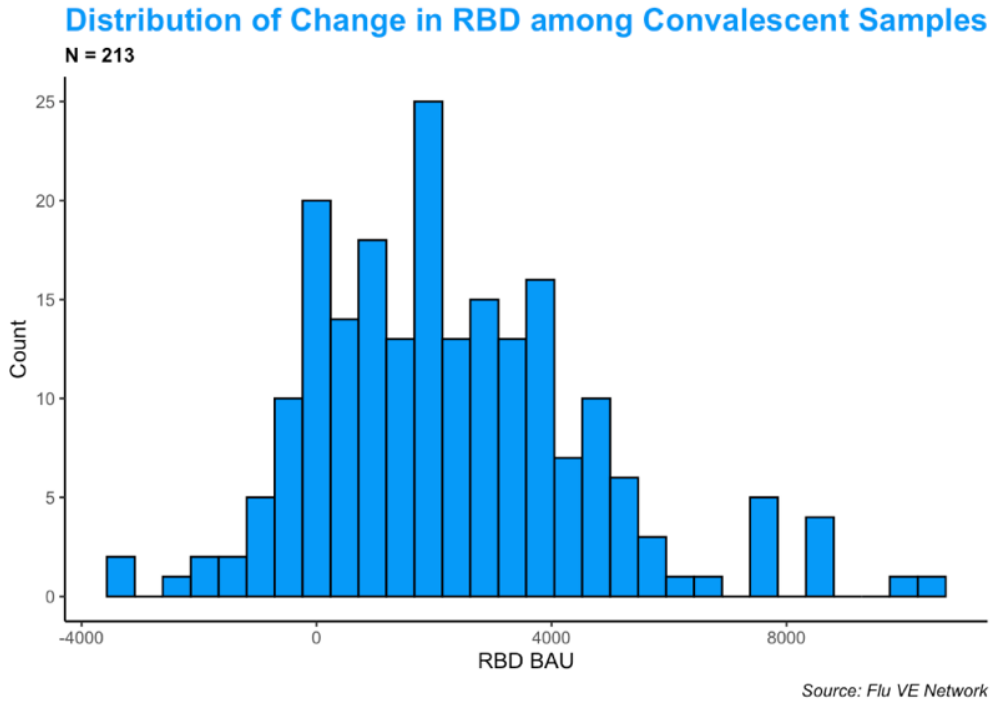


Figure 15 *Distribution of RBD among paired samples (BAU/ml)*

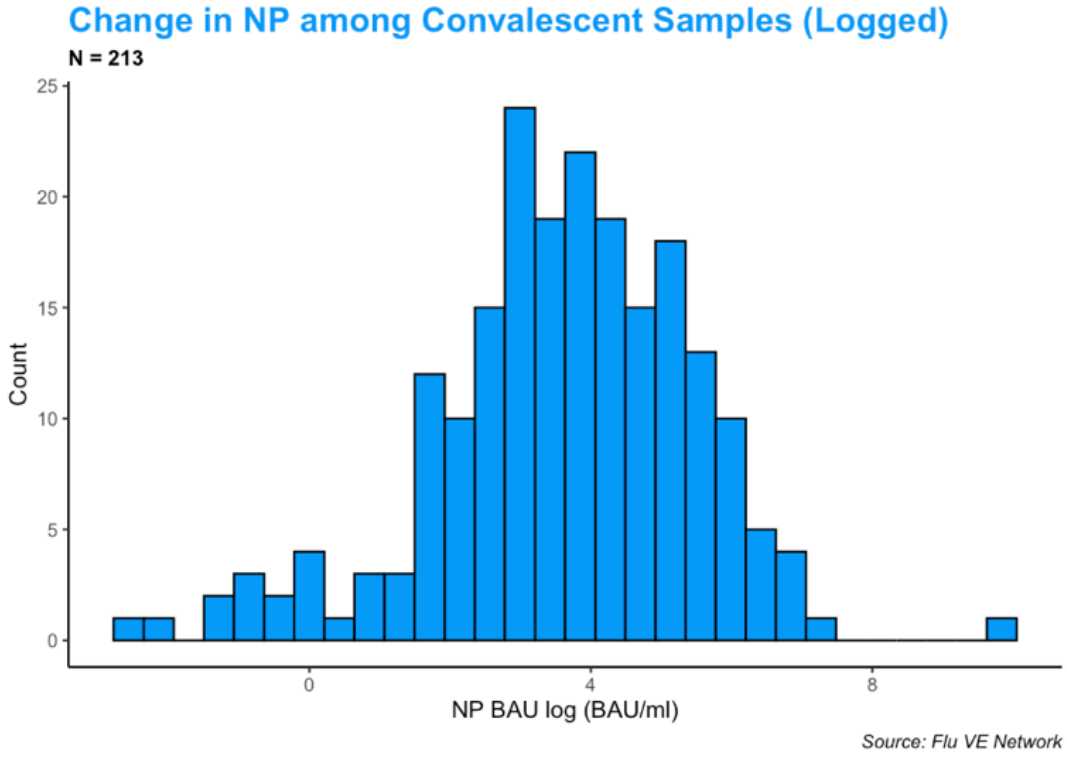


Figure 16 *Distribution of NP among paired samples*

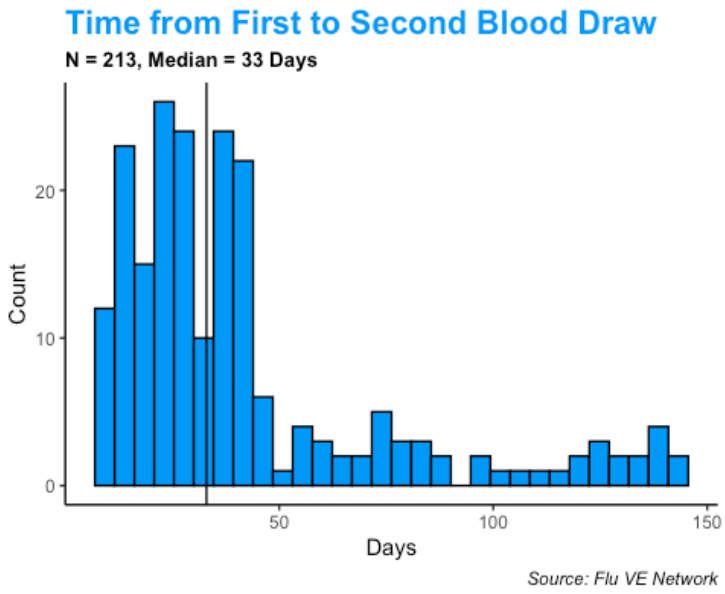


Figure 17 *Time between blood draws (right skewed)*

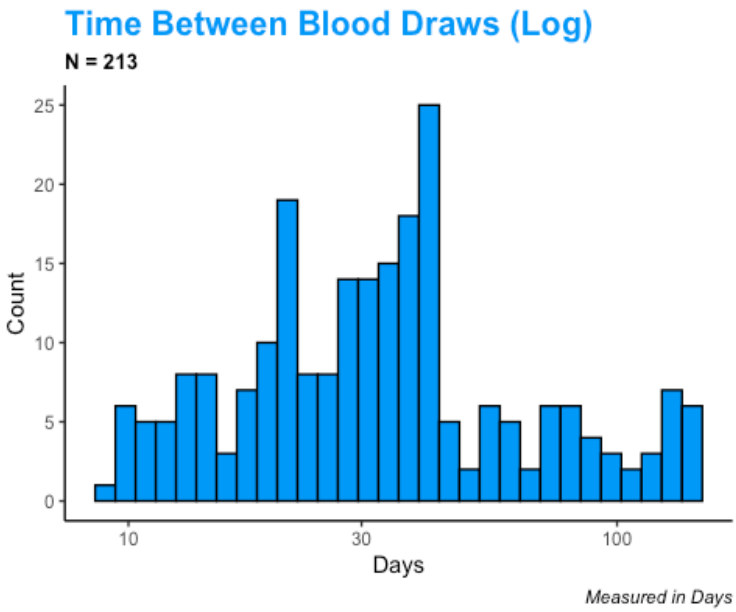


Figure 18 *Time between blood draws (logged) (days)*

As shown above, RBD changes were normally distributed (figure 15) while NP levels were skewed and required a log transformation (figure 16). Time between blood draws was right skewed (figure 17) and was log transformed (figure 18).

Known Prior Infection and Seropositivity (NP BAU/ml Cutoff of 6.9)

As mentioned previously, "Evidence of Prior Infection" is calculated from the MFI ratio cutoff of 1.2 from the Tetracore assay as a range for seropositivity. Evidence of prior infection is coded as a variable based on this cutoff and gives us insight into who recently had a natural infection in the past (figure 19). As mentioned above, some participants in the study arrived for a first blood draw 10+ days after their symptoms, showing signs that their NP antibody levels may have already been rising from their current infection. When conducting a basic sensitivity analysis, 74% of known prior lab confirmed infections were seropositive, but this may be due to time and varying time since immune event. We hypothesized that among these vaccinated individuals with a breakthrough infection, that those who had a prior COVID infection would have a more abrupt increase in NP BAU between the acute and convalescent draw because of an antibody boosting effect. As shown in figure 9, there does appear to be a difference in kinetics between those with evidence of prior infection compared to those without.

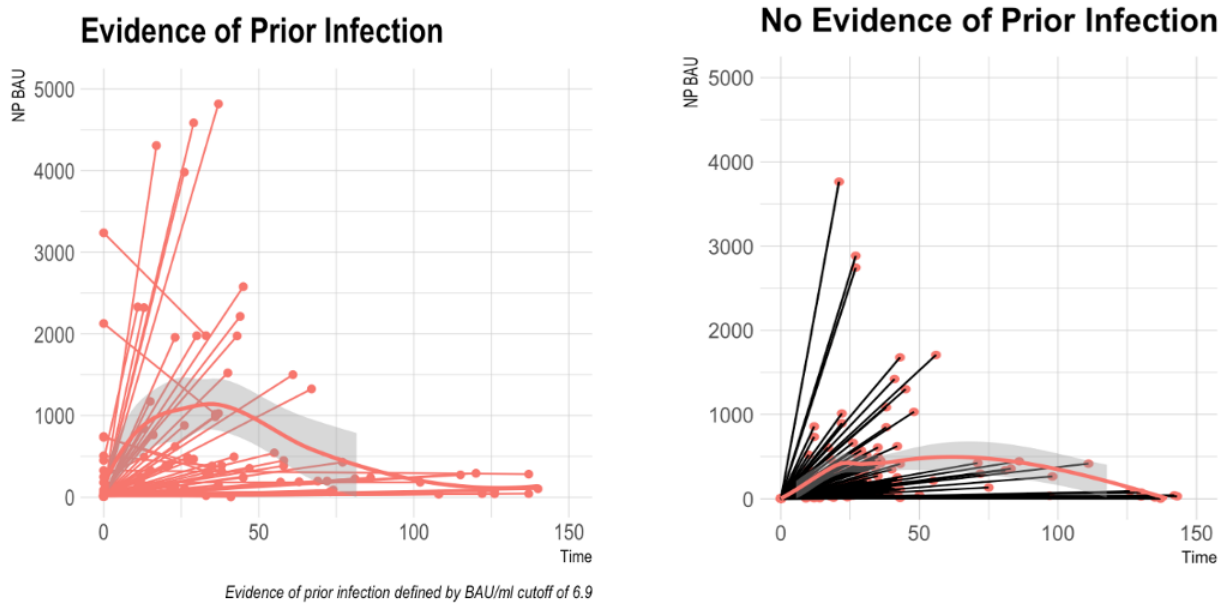


Figure 19 *Evidence of prior infection among paired samples: trend lines represent median of NP BAU/ml levels stratified by those who were serpositive*

Aim 2a: Change in NP/RBD BAU between acute and convalescent draw (outcome variable) as predicted by the baseline NP/RBD BAU level and adjusted for covariates.

We expect that NP level at acute draw will predict the amount of change in NP between visits, when controlling for demographic characteristics and time. Moreover, since antibodies to RBD could be present from both the vaccine and any prior infection, the change in RBD BAU is also of interest in relation to its level at baseline. Several models adjusted for covariates were examined. In the simplest model containing just age, sex and time between the acute and convalescent blood collection, RBD BAU at baseline had an inverse association with the change in RBD BAU between the acute and convalescent collection. This model explained 32.1% of the variance. Subsequent models also explained similar portion of the variance with the regression coefficient for baseline RBD BAU remaining negative. Specifically, higher RBD levels at baseline appear to be associated with a negative change (-0.84 [-1.05,-0.63]) after infection. For every 10 days increase between visits, on average, participants' RBD BAU/ml levels increased by 106.1 [98, 202.4]. For the similar analysis of NP change, models explained relatively little of the variance, though the regression coefficient was also negative.

Model 4: RBD Change Stepwise Comparison

RBD Change (BAU/ml), Step 1

	RBD Change(BAU/ml)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	3886.46	2835.13 – 4937.79	**<0.001**
Age(Years)	-0.00	-17.39 – 17.39	1.000
Sex: Male	-142.24	-692.43 – 407.94	0.611
Days Between Blood Draws	10.86	2.43 – 19.29	**0.012**
RBD at Baseline(BAU/ml)	-0.86	-1.04 – -0.68	**<0.001**
Observations	199		
R^2 / R^2 adjusted	0.321 / 0.307		

Table 9 *Step1 RBD change regression model*

RBD Change (BAU/ml), Step 2

	RBD Change(BAU/ml)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	3619.92	2118.13 – 5121.71	**<0.001**
Age(Years)	1.05	-16.59 – 18.69	0.906
Sex: Male	-129.35	-685.27 – 426.56	0.647
Days Between Blood Draws	11.14	2.32 – 19.97	**0.014**
RBD at Baseline(BAU/ml)	-0.84	-1.04 – -0.64	**<0.001**
Time Since Last Vaccination Dose	-0.52	-4.46 – 3.43	0.797
Vaccine Type(First Dose): PFIZER	416.57	-189.83 – 1022.97	0.177
Vaccine Type(First Dose): JANSSEN	-193.75	-1661.44 – 1273.93	0.795
Observations	197		
R^2 / R^2 adjusted	0.324 / 0.299		

Table 10 *: Step2 RBD regression model (adding vaccine type and time since vaccination)*

RBD Change (BAU/ml), Step 3

	RBD Change(BAU/ml)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	3346.48	1662.95 – 5030.00	**<0.001**
Age(Years)	3.67	-17.15 – 24.49	0.728
Sex: Male	-118.64	-715.33 – 478.06	0.695
Days Between Blood Draws	10.61	0.98 – 20.24	**0.031**
RBD at Baseline(BAU/ml)	-0.84	-1.05 – -0.63	**<0.001**
Time Since Last Vaccination Dose	-0.70	-5.04 – 3.65	0.752
Vaccine Type(First Dose): PFIZER	483.23	-171.86 – 1138.31	0.147
Vaccine Type(First Dose): JANSSEN	-75.83	-1614.79 – 1463.12	0.923
Health Condition(no/yes): Yes	-262.01	-1022.26 – 498.24	0.497
Days Between Symptom Onset and Enrollment	83.22	-96.85 – 263.29	0.363
Observations	183		
R^2 / R^2 adjusted	0.308 / 0.272		

Table 11: *Step3 RBD regression model (adding health condition and number of days since symptom onset)*

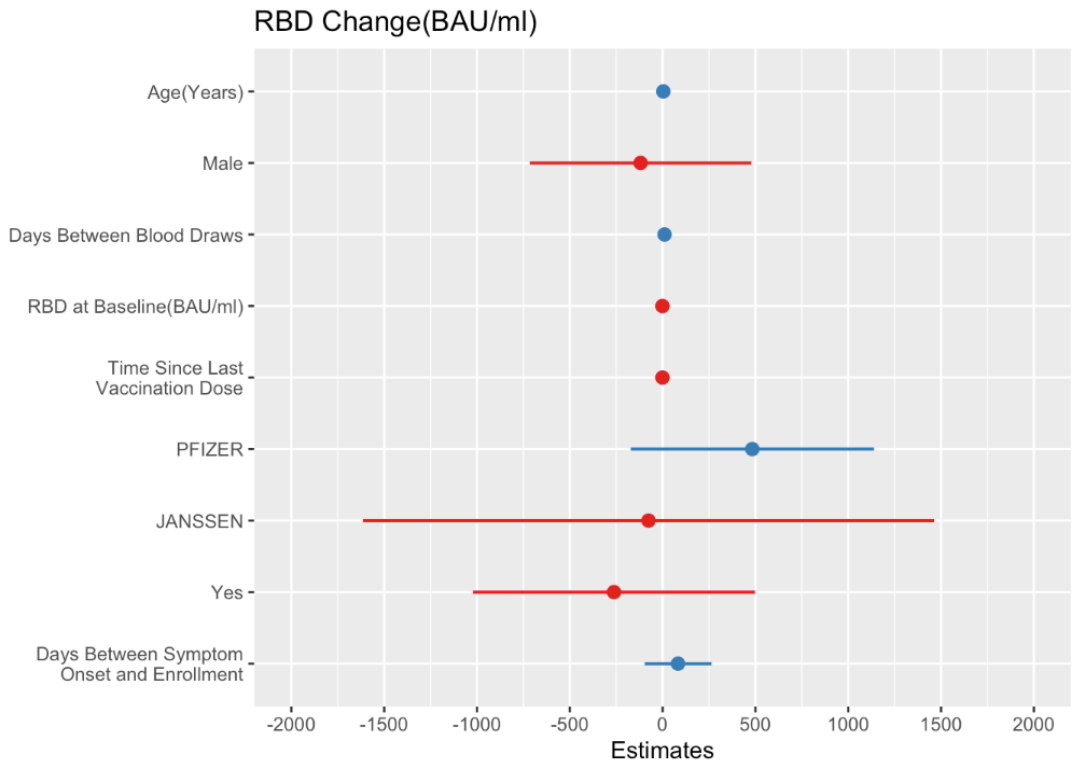


Figure 20 *Plot of Model 4*

We used a stepwise to find best model fit (Table 9,10,11). Among individuals who returned for a second blood draw, for every 100 increase in RBD BAU/ml at baseline, on average, RBD BAU/ml level changed on average by -84 (-105, -63) between blood draws, when controlling for age, sex days between blood draws, RBD at baseline, time since last vaccine dose, vaccine type, and days between symptom onset and enrollment (figure 20). For every ten day increase in time between blood draws, RBD increased, on average, by 106.1 (98, 202.4) when controlling for age, sex days between blood draws, RBD at baseline, time since last vaccine dose, vaccine type, and days between symptom onset and enrollment.

Model 5: NP Change Stepwise Comparison[4]

NP Change (BAU/ml), Step 1

	NP Change(BAU/ml)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	883.24	462.70 – 1303.77	**<0.001**
Age(Years)	-4.48	-11.76 – 2.81	0.227
Sex: Male	-37.75	-266.18 – 190.67	0.745
Days Between Blood Draws	-3.71	-7.21 – -0.21	**0.038**
NP at Baseline(BAU/ml)	-0.63	-1.03 – -0.24	**0.002**
Observations	199		
R^2 / R^2 adjusted	0.070 / 0.050		

Table 12: *Model 5, NP change by NP Baseline BAU/ml*

NP Change (BAU/ml), Step 2

	NP Change(BAU/ml)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	655.95	113.19 – 1198.70	**0.018**
Age(Years)	-3.76	-11.04 – 3.52	0.309
Sex: Male	-24.40	-251.34 – 202.53	0.832
Days Between Blood Draws	-3.20	-6.81 – 0.41	0.082
NP at Baseline(BAU/ml)	-0.61	-1.01 – -0.21	**0.003**
Time Since Last Vaccination Dose	0.13	-1.41 – 1.67	0.867
Vaccine Type(First Dose): PFIZER	180.35	-64.87 – 425.56	0.148
Vaccine Type(First Dose): JANSSEN	581.08	-11.85 – 1174.01	0.055
Observations	197		
R^2 / R^2 adjusted	0.094 / 0.061		

Table 13 *Stepwise Model 6 NP BAU/ml*

[4] Sex coded as 0 = Female, 1 = Male

NP Change (BAU/ml), Step 3

	NP Change(BAU/ml)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	676.41	68.25 – 1284.57	**0.029**
Age(Years)	-2.33	-10.72 – 6.06	0.584
Sex: Male	15.63	-222.71 – 253.97	0.897
Days Between Blood Draws	-3.05	-6.90 – 0.81	0.120
NP at Baseline(BAU/ml)	-0.54	-0.95 – -0.12	**0.011**
Time Since Last Vaccination Dose	0.47	-1.20 – 2.15	0.577
Vaccine Type(First Dose): PFIZER	209.11	-49.66 – 467.88	0.113
Vaccine Type(First Dose): JANSSEN	629.11	20.37 – 1237.84	**0.043**
Health Condition(no/yes): Yes	-122.89	-425.52 – 179.75	0.424
Days Between Symptom Onset and Enrollment	-64.81	-138.78 – 9.15	0.085
Observations	183		
R^2 / R^2 adjusted	0.122 / 0.077		

***Table 14**: Model 6 NP BAU/ml*

Interpretation of Model 5[5]

We also used a stepwise analysis to find the best model fit (table 12, 13,1 4). Using multiple linear regression, we found that NP at baseline level (BAU/ml) was associated with the change in NP BAU/ml between blood draws, when controlling for age, sex, time between visits, vaccine type, health condition and days between symptom onset and enrollment. Among paired individuals, individuals who were

[5] Vaccine type coded as 0 = Moderna, 1 = Pfizer, 2 = Janssen | Health condition coded as 0 = No, 1 = Yes

administered J&J for their first dose of vaccine had on average a 629.11 (20.37, 1237.84) higher NP BAU/ml level than those who had Moderna for their first dose, when controlling for age, sex, days between blood draws, NP at baseline, health condition and days between symptom onset and enrollment. For every 10 increase in NP BAU/ml level at baseline, on average, NP BAU/ml levels decreased by 5.4 (-9.5,-1.2) between first and second blood draws when controlling for age, sex, days between blood draws, vaccine type, health condition and days between symptom onset and enrollment.

Aim2b: Health Conditions (Model 6)

We can also examine whether worse health is associated with poor RBD and NP responses. We will started mapping with spaghetti graphs the change in titers between visits, stratified by health condition (figure 21). Then we conducted a multiple linear regression to further explore this relationship. As shown below, with a wide distribution of time, we are catching some of the antibody waning with the length of time between visits. This requires further use of the aforementioned time groups. As expected, immune response appears to be stronger in those without health conditions in the first 21 days. However,

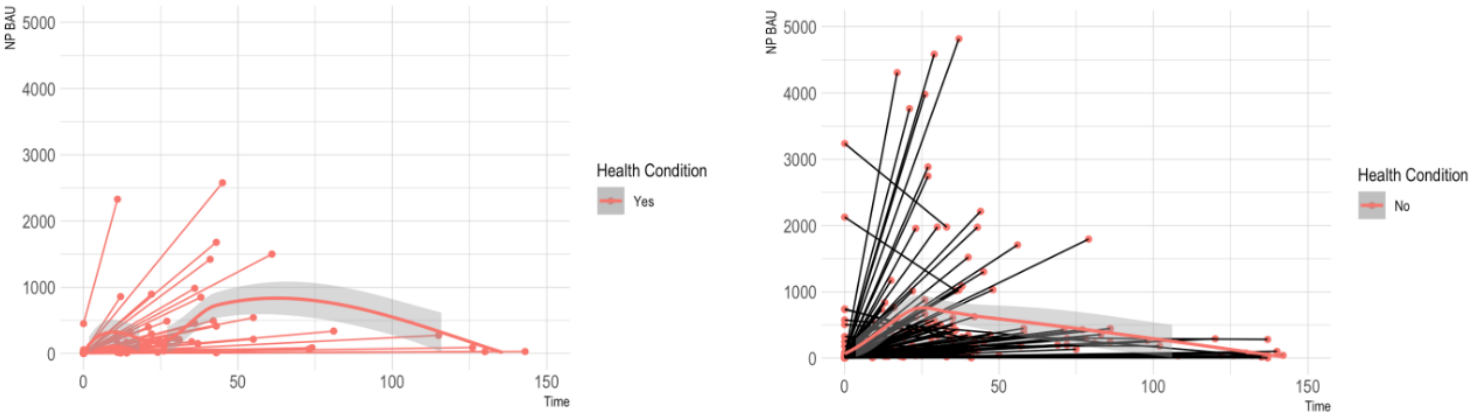


Figure 21 *Health conditions with median of those with and those without pre-existing chronic conditions (self reported)*

due to some interesting outliers, at peak antibody rise, on average, those with health conditions appear to have a similar if not stronger antibody response. Due to small sample size and waning, 65+ categories become less clear.

Age groups stratified by health condition did not appear to show any meaningful associations when exploring the data further (figure 22, 23,24). Interestingly, immune responses between age categories appeared to be similar (Table 15). The rise and expected waning of NP antibody levels was apparent when stratifying by time.

	NP Change Between Blood Draws(BAU/ml)
Predictors	*p*
Health Condition	0.622
age group	0.761
Time Categories(Days))	0.141
Residuals	
Observations	194
R^2 / R^2 adjusted	0.040 / 0.004

	RBD Change Between Blood Draws(BAU/ml)
Predictors	*p*
age group	0.124
Time Categories(Days))	**<0.001**
Residuals	
Observations	208
R^2 / R^2 adjusted	0.223 / 0.200

Table 15 *Simple ANOVA NP Change between blood draws compared to Health condition status*

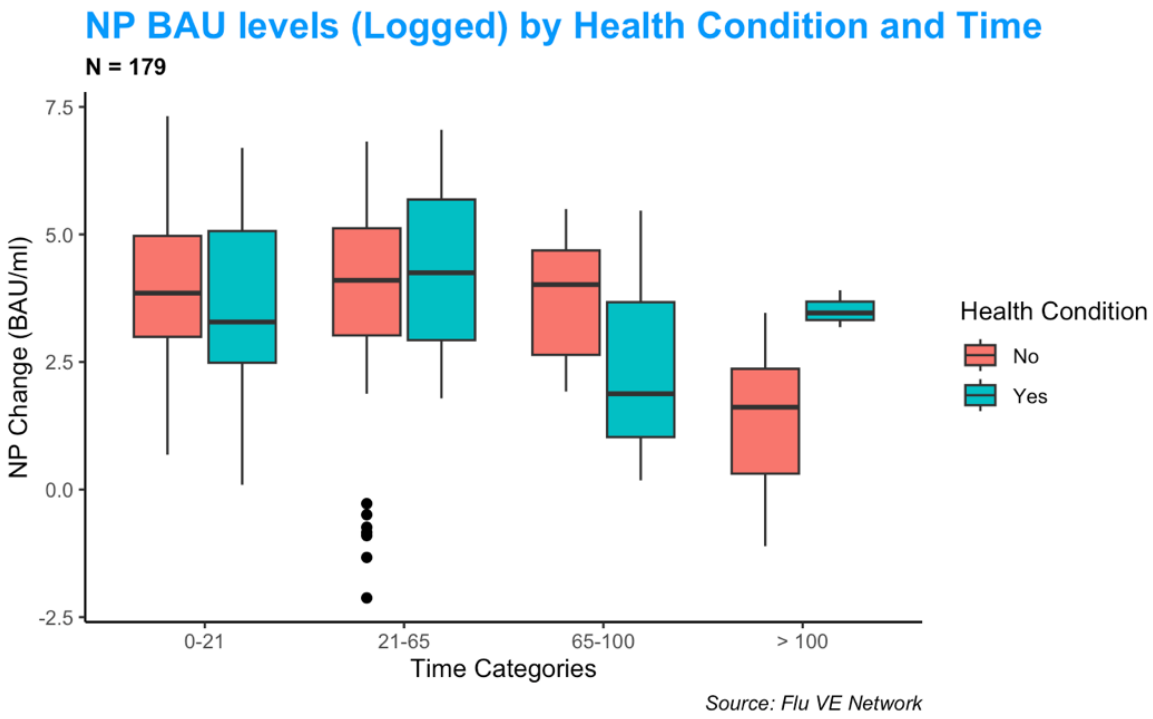


Figure 22 *NP BAU/ml Change by Age group*

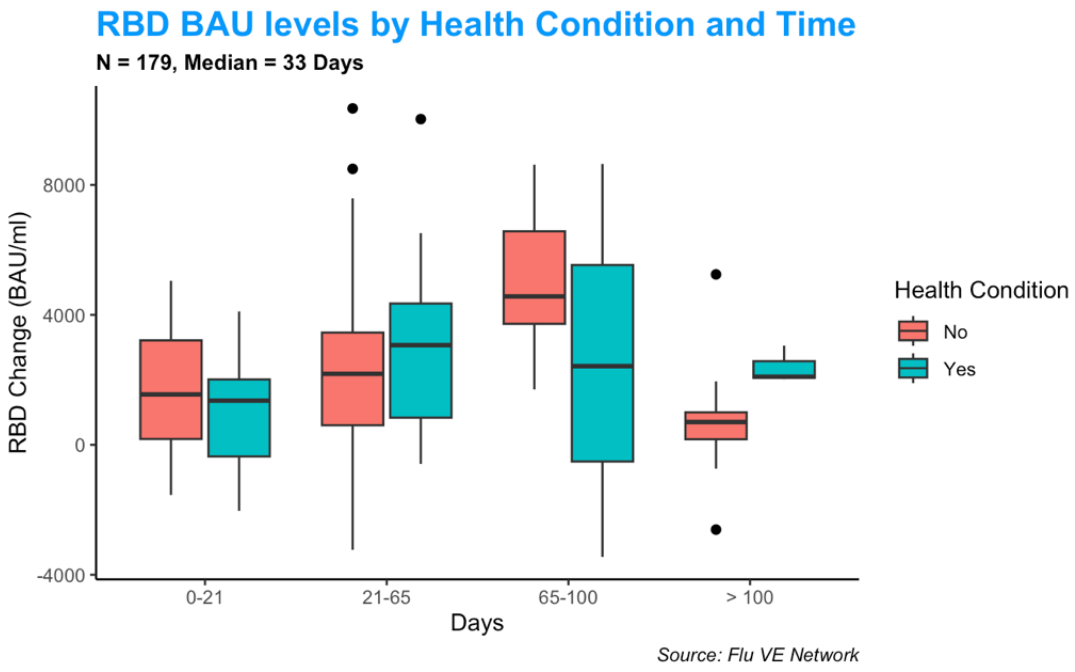


Figure 23 *RBD BAU/ml levels by Health Condition and time between visits*

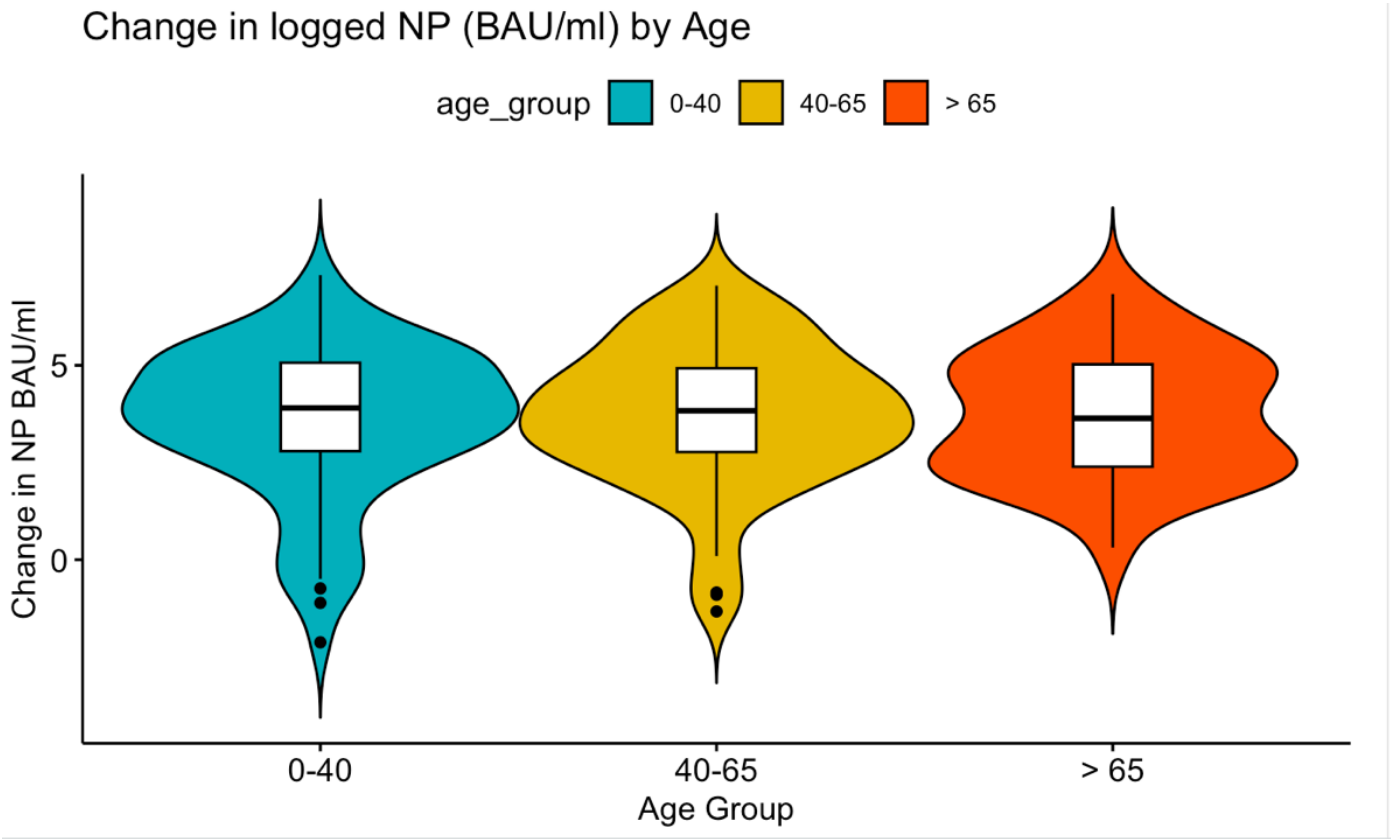


Figure 24: *Change in NP BAU/ml by Age Group*

We used logged NP change as our continuous outcome (BAU/ml) with our main predictor as Health Condition (referent: no health condition) (figure 25). We accounted for site variation, sex, age,

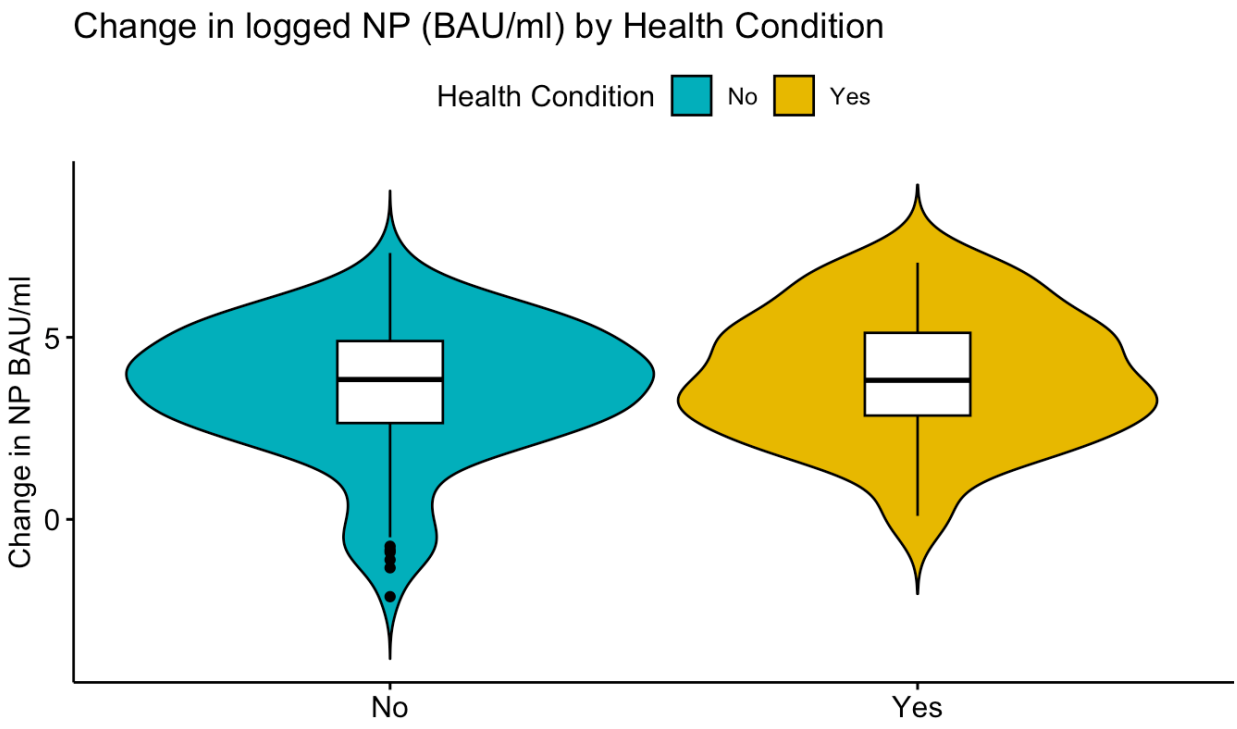
	Logged NP Change Between Blood Draws(BAU/ml)		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	4.55	3.58 – 5.52	**<0.001**
Health Condition(no/yes): Yes	0.08	-0.52 – 0.68	0.802
siteMI	0.67	-0.23 – 1.58	0.145
sitePA	2.38	0.68 – 4.07	**0.006**
siteTN	0.21	-1.29 – 1.72	0.780
siteTX	0.35	-0.59 – 1.29	0.463
siteWA	-0.12	-0.68 – 0.44	0.674
Sex: Male	-0.06	-0.54 – 0.42	0.796
Age(Years)	-0.00	-0.02 – 0.02	0.872
Days Between Blood Draws	-0.01	-0.02 – -0.00	**0.008**
Days Between Symptom Onset and Enrollment	0.01	-0.13 – 0.15	0.889
prev_inf_bauPositive	-1.46	-1.97 – -0.95	**<0.001**
Observations	190		
R^2 / R^2 adjusted	0.253 / 0.207		

Table 16 *Model 6: Multiple linear regression for logged NP Change BAU/ml and Health condition*

ed

individuals, there was no significance between logged NP change between blood draws (BAU/ml) and health condition status, when controlling for sampling site, sex, age, days between blood draws and evidence of prior infection (table 16). For every one increase in days between blood draws, logged NP change increased by -0.01 (-0.02,0.00), when controlling for sampling site, sex, age, days between blood draws and evidence of prior infection (prev_inf_bau). Those who were positive for prior infection by seropositivity and BAU/ml cutoff on average had a -1.46 (-1.97, -0.95) change in logged NP BAU/ml level hen controlling for sampling site, sex, age, days between blood draws.

Figure 25 *Change in NP BAU/ml by Health Condition*



Model 7: Follow Up at +10 Days

For those at 10+ days of follow, questionnaires were filled out, asking if they had fully recovered/whether they continued to experience symptoms. Of the 484 people who responded after 10 days, 63 had not "mostly recovered", while 403 had recovered. Eighteen were excluded or did not answer. Using a logit model, we examined whether there is an association between recovery and acute NP level at baseline, controlling for age, sex and time since symptom onset.

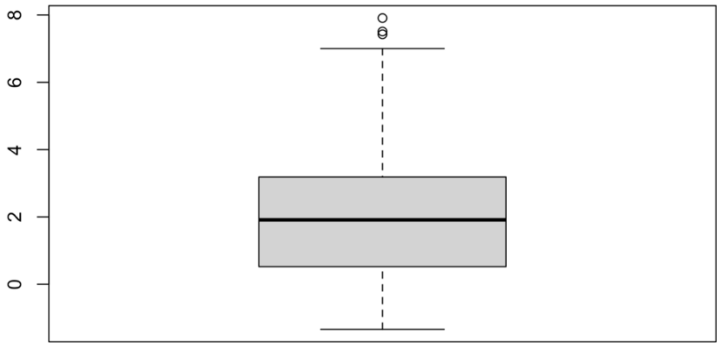


Figure 26 *Not Recovered after 10+ Days*

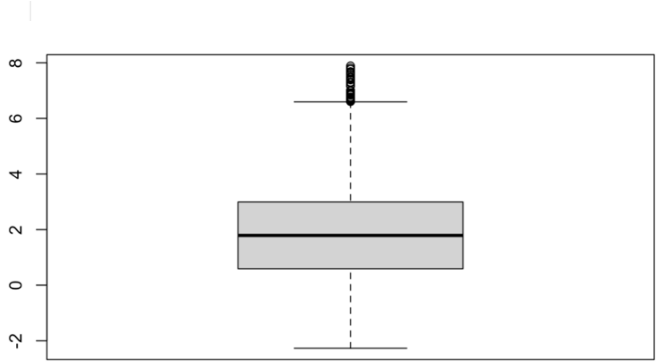


Figure 27 *Recovered after 10+ Days (NP BAU log at First Visit)*

This logistic regression model examines the relationship between long term recovery (yes or no) and NP at acute blood draw (BAU/ml). **There did not appear to be an association between NP level at acute**

	fol recover		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	3.97	2.74 – 5.79	**<0.001**
RBD at Baseline(BAU/ml)	1.00	1.00 – 1.00	0.494
Age(Years)	0.99	0.99 – 1.00	0.052
Sex: Male	1.36	1.07 – 1.74	**0.013**
Days Between Symptom Onset and Enrollment	0.98	0.92 – 1.04	0.469
Observations	1666		
R^2 Tjur	0.006		

Table 17

	fol recover		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	4.16	2.91 – 5.97	**<0.001**
NP at Baseline(BAU/ml)	1.00	1.00 – 1.00	0.767
Age(Years)	0.99	0.99 – 1.00	0.058
Sex: Male	1.35	1.06 – 1.73	**0.014**
Days Between Symptom Onset and Enrollment	0.98	0.92 – 1.04	0.473
Observations	1666		
R^2 Tjur	0.006		

Table 18

blood draw and recovery status after 10+ days (figure 26, figure 27). **The same was found with RBD BAU/ml.** With sex, however, the reference category is female, so the odds ratio of 1.36 (1.07, 1.74) suggests that, on average, males have 1.36 times higher odds of being recovered after 10+ days than females in the study group, after adjusting for the effect of other variables in the model (about a 10% percentage difference).

Results

When examining acute samples (N = 2,576), the RBD (BAU/ml) levels at baseline were significantly influenced by the number of vaccine doses. Four doses had the greatest effect (point estimate of 3306.46, 95% CI: 2537.97, 4074.96) followed by three doses (2371.79, 95% CI: 1752.80, 2990.78), and two doses (923.01, 95% CI: 306.35, 1539.66) when controlling for demographics and days between symptom onset and enrollment. The median of RBD values at blood draw was 1847.481. Age was found to be negatively associated with RBD, with a decrease of 73.8 (-119.5, -28) BAU/ml for every ten-year increase in age, when controlling for vaccination status, time since last dose, vaccine type, sex, race, and days between symptom onset and enrollment. Participants who received the Pfizer vaccine for their first dose had -325.46 RBD (95% CI: -475.12, -175.8) BAU/ml lower levels compared to those who received Moderna for their first dose. The effect estimates remained the same when adjusting for time since the last vaccine dose.

The study found no significant relationship between RBD level and COVID positivity among unvaccinated individuals after controlling for age, sex, and race. However, among unvaccinated individuals, the odds of testing positive for COVID-19 were 4.69 times higher for Asian participants compared to white participants (with a 95% confidence interval of 1.14 to 20.82), after controlling for RBD at baseline, age, sex, and race.

Among vaccinated individuals, those who were COVID-19 positive had -338.40 (95% CI: -494.01, -182.79) RBD BAU/ml level at baseline compared to those who were PCR negative and vaccinated ($p < 0.001$). Higher RBD levels were observed at baseline among individuals who received three doses (1272.23, 95% CI: 1085.03, 1459.43) and four doses (2072.47, 95% CI: 1571.90, 2573.04) compared to those who received two doses. Age was found to be negatively associated with RBD among vaccinated individuals, with a decrease of 52.7 (-98.5, -7.0) RBD BAU/ml for every ten-year increase in age, when controlling for age, sex, race, and time since the last vaccination dose. RBD at baseline was

found to decrease by -1.65 (95% CI: -2.57, -0.73) for every one-day increase between time since the last vaccination dose, when controlling for vaccine status, race, age, and sex.

Among individuals who returned for a second blood draw, RBD levels at baseline were negatively associated with the change in RBD levels over time. For every 100 increase in RBD BAU/ml at baseline, RBD BAU/ml level changed on average by -84 (95% CI: -105, -63) between blood draws, when controlling for age, sex, days between blood draws, RBD at baseline, time since the last vaccine dose, vaccine type, and days between symptom onset and enrollment. RBD at baseline was found to increase by 106.1 (95% CI: 98, 202.4) for every ten-day increase in time between blood draws, when controlling for age, sex, days between blood draws, RBD at baseline, time since the last vaccine dose, vaccine type, and days between symptom onset and enrollment.

Model 5 showed that individuals who received the Johnson & Johnson vaccine for their first dose had significantly higher NP antibody levels compared to those who received Moderna, with an average increase of 629.11 (20.37, 1237.84) NP BAU/ml when controlling for other factors. Additionally, for every 10 increase in NP BAU/ml level at baseline, there was an average decrease of 5.4 (-9.5, -1.2) NP BAU/ml levels between the first and second blood draws, after controlling for other variables such as age, sex, days between blood draws, vaccine type, health condition, and days between symptom onset and enrollment. This suggests that the choice of vaccine may play a role in antibody levels, and monitoring antibody levels over time may be important for maintaining immunity.

There was no significant relationship found between logged NP change between blood draws (BAU/ml) and health condition status among paired individuals, after controlling for sampling site, sex, age, days between blood draws and evidence of prior infection (prev_inf_bau). For every one increase in days between blood draws, logged NP change increased by -0.01 (-0.02, 0.00), after controlling for the aforementioned variables. Individuals who tested positive for prior infection by seropositivity and BAU/ml cutoff, on average, had a -1.46 (-1.97, -0.95) change in logged NP BAU/ml level when controlling for sampling site, sex, age, and days between blood draws.

A sub-analysis examined the relationship between long-term recovery and NP/RBD levels at acute blood draw, and sex. There was no association between NP/RBD levels and recovery status after 10+ days, but the odds ratio of 1.36 (1.07, 1.74) suggests that, on average, males have 1.36 times higher odds of being recovered after 10+ days than females in the study group, after adjusting for other variables in the model.

Discussion

This thesis filled several important gaps in the literature on COVID-19 antibody responses as measured by RBD and NP BAU levels. In aim1b, I concentrated on individuals who had received at least two SARS-CoV-2 vaccines. I found that having a breakthrough infection, as indicated by a positive COVID-19 PCR test at the acute visit, was associated with a significantly lower level of anti-RBD antibody. This result was significant after controlling for key demographic factors as well as the time between the last vaccine and the acute visit, and the number of vaccine doses. Hence, this implies that vaccinated individuals with a more robust or persistent anti-RBD response are relatively protected from breakthrough infection compared to those individuals with a less robust response. Since this analysis controlled for the number of vaccine doses, we cannot determine from this analysis the contribution of differences in vaccine number to this effect, which could be further explored in analysis stratified by vaccine number.

As expected, median levels of NP BAU began to spike if more than 9 days of symptomatic infection had past prior to first blood draw. Number of vaccine doses also predicted higher level of RBD BAU/ml levels.

This study also supplied some unique insights derived from blood collected at the time of acute illness presentation and during the convalescent phase of the illness. We found that there was a negative correlation between the level of antibodies at the time of acute symptoms and the increase in antibodies over the subsequent days. This is unexpected, as one would expect the immune response to continue to

increase (at least over this interval soon after infection). This negative correlation could be due to a ceiling effect, where the immune response has already reached its maximum level, or due to the fact that this aim only considered individuals who were vaccinated and infected and did not compare them to those who were unvaccinated and infected. This could lead to higher baseline levels of antibodies in the vaccinated individuals, which would make further increases more difficult to detect.

We also found no association between age and IgG antibody response. The same was true for health condition status. As discussed in the introduction, these variables continue to be explored as we seek to understand what determines severity of COVID-19 infection. These null findings may continue to show the need for better understanding of asymptomatic infections, or the evolution of variants as COVID-19 becomes more and more endemic in the US.

There are significant limitations to this cross-sectional dataset, but the odds ratios for likelihood of having a positive test were interesting to explore. We found that Hispanic participants had a higher likelihood of testing positive for COVID-19 compared to white participants, and that age was positively associated with COVID-19 positivity. Age and time since last vaccination dose were negatively associated with RBD BAU/ml level. The type of vaccine received was also associated with RBD BAU/ml level. Lastly, the study found that there was no association between recovery and acute NP level at baseline, after controlling for age, sex, and time since symptom onset.

We also examined the relationship between RBD and NP antibody levels and COVID-19 vaccination and infection. The results indicate that, at acute blood draw, the number of vaccine doses had a significant influence on RBD (BAU/ml) levels at baseline, with four doses having the greatest effect, followed by three doses and two doses. Age was negatively associated with RBD levels, with older individuals having lower levels. The choice of vaccine may also play a role in antibody levels, with individuals who received the Pfizer vaccine for their first dose having lower RBD levels than those who received Moderna. Among unvaccinated individuals, Asian participants had higher odds of testing

positive for COVID-19 compared to white participants, after controlling for RBD at baseline, age, sex, and race.

Future studies examining NP and RBD antibody levels should consider using a linear scale to make inferences about the immune response. A longitudinal strategy with better sampling than a cross-sectional design should be implemented to control for more variability in each model. However, the 7plex assay used in this study had no targets set to identify between different variants for each case, which limits the association between variants and antibody titer levels. Misclassification of some convalescent samples as paired samples due to plate re-runs was accounted for in the merged data. Participants in the study were administered different types of COVID-19 tests, and stratification was used to account for differences in vaccine criteria between the J&J vaccine and mRNA vaccines like Moderna and Pfizer. Those who tested positive for antigen tests but negative for PCR were excluded. Study participants were administered COVID-19 tests at both clinical and lab research sites.

We also examined the relationship between long-term recovery and NP/RBD levels at acute blood draw and sex. There was no association between NP/RBD levels and recovery status after 10+ days, but males have higher odds of long-term recovery.

Conclusion: Public Health Implications

In conclusion, we found that NP and RBD antibodies did not vary significantly by age group, and that NP and RBD levels at acute blood draw were predictors of respective antibody change over time. These findings provide important insights into the dynamics of the immune response to SARS-CoV-2. Additionally, our study identified higher RBD levels do not predict a stronger positive immune response. Looking ahead, the ongoing COVID-19 pandemic provides an opportunity to assess how antibody responses are changing with vaccination in the United States. Finally, our study also standardizes data on a linear scale by utilizing the Binding Antibody Unit conversion, which allows for more nuanced analyses and comparisons across studies. Utilizing this approach, we can examine the relationship between IgG

antibody binding to SARS-CoV-2 target proteins and various factors, such as vaccine status and health variables, to gain deeper insights into the immune response to SARS-CoV-2.

Appendix A

NP at Baseline Predicting NP Change (logistic regression)[6]

We examine this same association, but with evidence of prior infection from NP cutoff as the outcome, and NP change between visits as the main exposure. This logistic regression model examines the relationship between evidence of previous infection (positive or negative) and a continuous predictor variable (NP change). The odds ratio of 0.57 suggests that, on average, for each one-unit increase in NP logged change, the odds of having evidence of previous infection are 43% less likely, after adjusting for the effect of other variables in the model. In other words, those who have a higher NP BAU/ml change are less likely to [illegible] cutoffs.

	prev inf bau							
Predictors	*Odds Ratios*	*std. Error*	*std. Beta*	*standardized std. Error*	*CI*	*standardized CI*	*Statistic*	*p*
(Intercept)	4.13	2.93	0.49	0.13	1.06 – 17.33	0.29 – 0.82	2.00	**0.046**
NP Change(BAU/ml)Logged	0.57	0.06	0.37	0.07	0.46 – 0.70	0.25 – 0.54	-5.07	**<0.001**
Sex: Male	0.97	0.32	0.97	0.32	0.50 – 1.84	0.50 – 1.84	-0.10	0.920
Race: Black	0.59	0.72	0.59	0.72	0.03 – 5.53	0.03 – 5.53	-0.43	0.665
Race: Asian	1.60	0.71	1.60	0.71	0.66 – 3.83	0.66 – 3.83	1.06	0.289
Race: Other	0.66	0.46	0.66	0.46	0.15 – 2.45	0.15 – 2.45	-0.60	0.549
Race: Hispanic	1.17	0.55	1.17	0.55	0.46 – 2.90	0.46 – 2.90	0.34	0.735
Age(Years)	1.00	0.01	0.96	0.16	0.98 – 1.02	0.69 – 1.33	-0.25	0.802
Observations	197							
R^2 Tjur	0.167							

Figure 1: Model 6.a Evidence of Previous Infection compared to NP Change (BAU/ml)

NP BAU/ml change was logged in order to normalize the data (figure 28, 29). This logistic regression model examines the same relationship between evidence of previous infection (positive or negative) and a continuous predictor variable (NP change) but the differences of the logs between acute and convalescent

[6] Evidence of Previous Infection (prev inf bau) was coded as 0 being no evidence of prior infection and 1 being evidence of prior infection (Using NP BAU/ml cutoff of 6.9 for seropositivity)

blood draws. The odds ratio of 0.57 suggests that, on average, for each one-unit increase in NP change, the odds of having evidence of previous infection decrease by 43% (1 - 0.57 = 0.43), after adjusting for the effect of other variables in the model.

Justification for logged NP due to normalization:

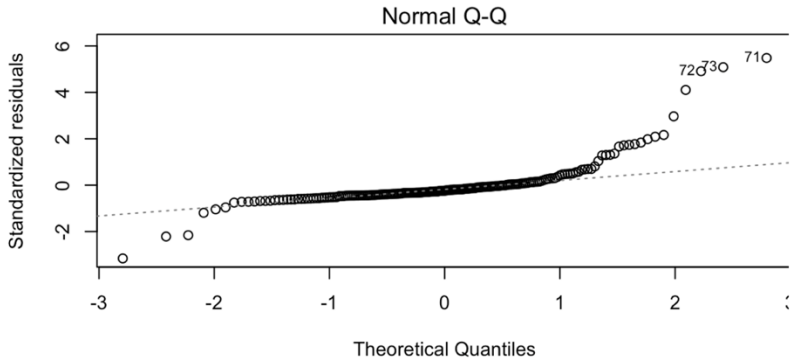


Figure 28 NP change not logged

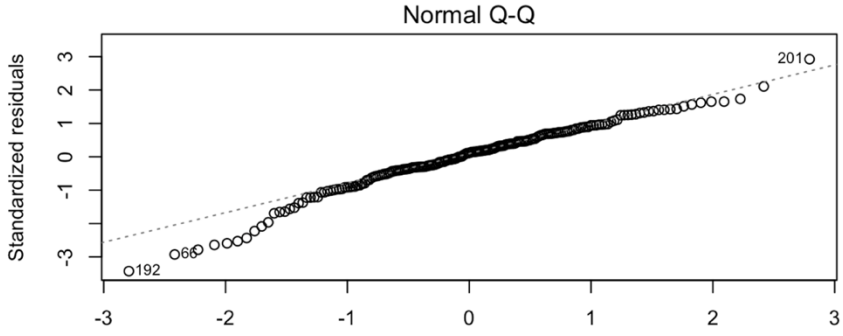


Figure 29: NP change logged

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