Strong Heart Study – Dakota Center

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Please find attached one (1) Manuscript for tribal review and approval. The intended journal is JAMA.

Manuscript~~~

Kim J. #713 **Predictors of SARS-CoV-2 Spike 1 Antibody Response Among Vaccinated US Adults: the C4R Study**

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Lay Summary

COVID-19 was one of the three leading causes of death in the US in 2020 and 2021. COVID-19 vaccines generate antibodies that can reduce the risk of infection and severity of illness. However, there may be some people who do not have an adequate increase in their antibodies after vaccination. We used data from the C4R cohort (which includes the Strong Heart Study) to investigate factors that may be associated with lower antibody levels after vaccination. We found that certain risk factors like older age, male sex, diabetes, and cigarette smoking are linked to lower antibody levels. We also found that a prior history of infection and certain types of vaccines are associated with higher antibody levels. Our research suggests certain people may not have as high of antibody levels after vaccination and we need to work on strategies to help these individuals.

Predictors of SARS-CoV-2 Spike 1 Antibody Response Among Vaccinated US Adults: the C4R Study

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Conflicts of Interest:

Keywords: COVID-19, vaccination, serosurvey, immunology, cohort study

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ABSTRACT

Importance: COVID-19 vaccines reduce the risk of severe infection by generating antibodies to the SARS-CoV-2 spike-1 (S1) protein. Lower anti-S1 antibody levels, compared to higher levels, are expected to provide less immunologic protection from SARS-CoV-2 infection.

Objective: Identify risk factors for lower anti-S1 antibody response following COVID-19 vaccination in a multi-ethnic U.S. population-based sample of adults.

Design, Setting, and Participants: The Collaborative Cohort of Cohorts for COVID-19 Research (C4R) is a national prospective cohort study of participants in 14 NIH-funded longitudinal cohort studies. This analysis includes C4R participants who self-reported one or two doses of an approved COVID-19 vaccine prior to participation in the C4R serosurvey from January 2021 through August 2022.

Exposures: Socio-demographic, anthropometric, and clinical risk factors were harmonized from pre-pandemic questionnaires, examinations, laboratory data, and events ascertainment. Pandemic-era risk factors were classified by C4R questionnaires and COVID-related events ascertainment.

Main Outcomes and Measures: Anti-S1 antibodies were measured from dried blood spots using Luminex-based microsphere immunoassays and reported as mean fluorescence intensity (MFI). Multivariable-adjusted linear and generalized additive regression models examined associations between risk factors and log-transformed anti-S1 MFI.

Results: Of 6797 participants, mean age was 73 years (range, 21-100), 59% were women, 77% were non-Hispanic white, 17% were Black, and 6% reported other race/ethnicity. 50% received the BNT162b2 (Pfizer) mRNA vaccine 48% received the mRNA-12753 (Moderna) mRNA vaccine. 17% self-reported natural infection with SARS-CoV2-2 prior to serosurvey participation. Anti-S1 antibody levels were highest at approximately 2 months following the first vaccine dose and then declined at an average rate of 23% (95%CI 20.9-24.9) per month. Age < 80 years and the mRNA-1273 vaccine were associated with higher anti-S1 antibody levels and a slower rate of decline in antibody levels over time. Other risk factors for lower anti-S1 antibody levels, but not rate of decline, were male sex, cigarette smoking, diabetes, body

mass index ≥ 35 kg/m^2, and 1 (versus 2) vaccine doses. Prior COVID-19 infection was associated with higher anti-S1 antibody levels.

Conclusions and Relevance: Adults with certain socio-demographic and clinical characteristics may have less robust antibody responses to COVID-19 vaccination. These individuals may be at greater risk of adverse COVID-19 outcomes and could be prioritized for re-vaccination.

INTRODUCTION

COVID-19 was one of the three leading causes of death in the US in 2020 and 2021.[1] One of the most important public health interventions for the pandemic has been COVID-19 vaccination, which significantly reduces risk of COVID-related hospitalization and death.[2,3] The primary mechanism of the most commonly used messenger RNA vaccines, the BNT162b2 (Pfizer) and mRNA-12753 (Moderna) vaccines, is production of antibodies against the spike protein of SARS-CoV-2.[4] Although mRNA vaccine-induced antibody production is generally robust, some studies have shown differential antibody responses to vaccination and waning antibody levels over time.[5-7] Variation in vaccine-induced antibody response may be clinically significant since lower humoral responses to SARS-CoV-2 vaccines are predictive of higher risk for breakthrough infections and for severe COVID-19 clinical outcomes in certain populations.[6,8-10] Hence, identification of individuals at risk of lower vaccine-induced antibody response and greater antibody waning could inform vaccination strategies based on personalized risk.

Most prior studies on vaccine immunogenicity have leveraged clinical trials or highly selected cohorts, such as health-care workers or residents of long-term care facilities.[6,11-13] A study conducted in the United Kingdom (UK) observed that lower rates of post-vaccination seropositivity were observed in older adults, men, and those with chronic health conditions.[5,14] Nonetheless, the determinants of post-vaccination antibody response, including magnitude and durability of antibody levels, has not been comprehensively investigated in multi-ethnic, diverse, population-based U.S. sample.

This study aimed to identify risk factors for lower anti-S1 antibody levels after COVID-19 vaccination in the Collaborative Cohort of Cohorts for COVID-19 Research (C4R) cohort.[15] C4R is a national prospective study of US adults participating in 14 longitudinal cohort studies that collectively constitute a large, well-characterized, population-based sample of Americans and that reflect the racial, ethnic, socioeconomic, and geographic diversity of the United States. Anti-S1 antibody levels were measured by serosurvey and examined with respect to pre-pandemic and pandemic-era socio-demographic, anthropometric, and clinical factors. Determinants of peak antibody levels and rate of antibody decline over time-since vaccination were explored.

METHODS

Study Participants

In order to study the impact of the COVID-19 pandemic on US adults, C4R aimed to perform standardized assessment of COVID-19 illness in participants in 14 constituent cohorts, as previously described.[15] All cohort participants who were alive on March 1, 2020, and had not withdrawn consent for cohort participation were considered eligible for enrollment in the C4R. Across this population, C4R ascertained SARS-CoV-2 infection and COVID-19 illness using standardized questionnaires, ascertainment of COVID-related hospitalizations and deaths, and a SARS-CoV-2 serosurvey conducted via dried blood spots (DBS). Institutional review board approval was obtained from all study sites. Informed consent was obtained from each study participant.

Inclusion criteria for the present study were self-report of one or two vaccinations for COVID-19 prior to submission of a valid DBS for serology assay. We excluded participants who reported, or were eligible for, a third vaccination prior to their date of DBS completion (Figure 1).

C4R Serosurvey

The C4R serosurvey was accomplished via DBS, as previously described.[15,16] Briefly, the DBS requires that several drops of whole blood, from a finger prick or blood collection tube, be absorbed into a specially designed card. Participants who consented to the serosurvey completed the DBS at an in-person exam or at home, following detailed instructions. The present study includes DBS collected between April 2021 and August 2022.

Serology assays were performed on completed DBS by the New York State Wadsworth Center Laboratory (Albany, NY, USA) using validated methods.[16] The assays were designed to detect IgG for SARS-CoV-2 S1 protein, which may be induced by natural infection or currently approved COVID-19 vaccines, or nucleocapsid (N) protein, which is induced by natural infection only. Briefly, Magplex-C microspheres (Luminex Corp., Austin, TX, USA) with different bead regions coupled to the SARS-CoV-2

S1 and N antigens (Sino Biological, Wayne, PA, USA) were used. Mean fluorescence intensity (MFI) was analyzed using a FlexMap 3D instrument (Luminex Corp., Austin, TX, USA). Five separate bead sets were used over the period of analysis.

Antibody response was classified as reactive or non-reactive based on the mean and standard deviation (SD) of anti-S1 MFI values in uninfected (pre-pandemic) DBS samples.[16] For each bead set, the reactivity threshold was calculated as the mean + 6 SD of the uninfected (pre-pandemic) MFI. Samples above this threshold were classified as reactive.

C4R Questionnaires

C4R collected information on COVID-19 infection and vaccination status, as well as other pandemic-era characteristics, via two waves of questionnaires conducted from April 2020 through August 2022. Questionnaires were administered either in-person or via telephone, online portal, or mail. Participants were asked regarding COVID vaccination status, date of first vaccine administration, number of vaccines received, and vaccine manufacturer. Time-since-vaccination was defined as days from first vaccination to DBS completion. COVID-19 infection status and date of infection were defined by self-report.

Pre-pandemic Measures

C4R cohorts have performed longitudinal data collection on their participants for up to 51 years of follow-up via in-person examinations, follow-up calls, and/or mail. These data were harmonized across cohorts by C4R, as previously described.[15] Age, sex, and educational attainment were self-reported. Race and ethnicity were self-reported and categorized according to the 2000 Census methods; these characteristics were explored as potential risk factors due to observed differences in COVID-19 infection rates and clinical outcomes by racial/ethnic groups.[17,18] Smoking status was self-reported as never or ever, defined as lifetime consumption of at least 100 cigarettes. Among ever-smokers, former or current smoking status (defined as smoking within the past 30 days) was self-reported, with biochemical confirmation using

serum or urine cotinine in a subset. Height, weight, blood pressure, fasting lipids and blood glucose were measured using standardized protocols at the most recent in-person exam in each cohort. Hypertension was defined as a systolic blood pressure of 140 or higher, diastolic blood pressure of 90 or higher, or use of antihypertensive medications. Diabetes was defined as fasting blood glucose level of 126 mg/dL or greater or use of insulin or hypoglycemic medications. Chronic kidney disease was defined based on estimated glomerular filtration rate (eGFR) <45 mL/min/1.73m^2. Cardiovascular disease, asthma, and chronic obstructive pulmonary disease were defined based on self-report or by the occurrence of relevant clinical events over cohort follow-up.

Statistical Analysis

The difference in log-transformed anti-S1 MFI according to the candidate risk factors was estimated using multivariable linear regression models, which were adjusted for time-since-vaccination and laboratory batch number, to account for inter-batch differences. For interpretability purposes, we exponentiated the beta coefficient in order to present the results as mean percent difference in anti-S1 level. Multiple imputation was used to account for missing covariables in our regression model. Complete case analysis was performed as a sensitivity analysis.

Generalized additive models were used to examine changes in log-transformed anti-S1 MFI over time-since-vaccination. Associations between risk factors and rate of antibody decline were explored graphically by plotting predicted antibody level over time in strata of risk factors. Consistent with clinical trial data using approved vaccine schedules, our results suggested a maximum antibody response approximately 60 days after the receipt of the first vaccine dose.[5,7] We therefore tested the association of risk factors with the slope of the linear spline for the period ≥60 days-since-vaccination. The beta coefficient of interest was the interaction term "risk factor × slope of time from vaccine to DBS (per 30 days)." The beta coefficient was expontentiated and positive and negative coefficients were interpreted as slower and more rapid decline in anti-S1 levels, respectively.

All analyses were conducted in R Code (R Statistical Foundation, Vienna, Austria) on the C4R Analysis Commons[15]. Two-sided p-values less than 0.05 were considered statistically significant.

RESULTS

Participant characteristics

There were 6797 participants with anti-S1 antibody levels measured after 1 or 2 COVID-19 vaccinations (**Figure 1**). The mean time between vaccine dose and DBS collection was 3.9 months (SD 1.8; range 0-7.1).

Table 1 shows participant characteristics according to quartile of anti-S1 level. Mean age was 73 years (range, 21-100) and 75% of participants were aged 65 years or older, 59% were women, 77% were non-Hispanic white, 17% were Black, and 6% reported other race/ethnicity. Fifty percent received the BNT162b2 (Pfizer-BioNTech) mRNA vaccine and 48% received the mRNA-12753 (Moderna) mRNA vaccine. There were 17.6% of C4R participants who self-reported natural infection with SARS-CoV2-2 prior to serosurvey participation and 5.3% self-reported natural infection after vaccination but before serosurvey participation (**Table 1**).

The highest quartile of anti-S1 levels showed higher proportions of women, younger adults, adults with a history of prior COVID-19 infection, and recipients of the mRNA-1273 vaccine.

Antibody reactivity

Antibody reactivity was observed overall in 97% of vaccinated participants. The highest proportion of participants with anti-S1 reactivity was at 2-4 months between vaccine dose and DBS (**Table 2**). As the time between vaccine dose and DBS lengthened, non-reactivity was more prevalent among those who received the BNT162b2 vaccine compared with mRNA-1273 and those 80 years or older compared with younger age groups (**Table 2**). Similar trends persisted over time between vaccine dose and DBS measurement.

Risk factors for lower antibody response

Associations of candidate risk factors are shown in **Table 3**. Significant associations were observed for vaccine-related, socio-demographic, and other clinical factors. Compared to the BNT162b2 (Pfizer-BioNTech) vaccine, receipt of the mRNA-1273 vaccine was associated with a 94.4% (95% CI 84.2-105.4) higher anti-S1 response. A single vaccine dose compared with two doses was associated with a 39.0% (31.9-45.5) lower anti-S1 level. Age greater than 80 years was associated with a 30.4% lower anti-S1 level (95% CI, 18.5-40.6) compared with age less than 50 years. Men demonstrated a 20.4% lower antibody response (95% CI, 15.7-24.8) compared to women. Self-reported Asian race was associated with greater antibody response, although the confidence interval was wide in the setting of relatively small sample sizes in this group. Health behaviors and comorbidities were also associated with differential post-vaccination antibody response in fully adjusted models. Compared with never-smokers, lower antibody responses were observed in former smokers (6.7%, 95% CI, 0.9-12.2) and current smokers (15.2%, 95% CI, 5.9-23.7). Similar to current smoking, diabetes was associated with a 15.3% (95% CI 8.8-21.4) lower anti-S1 response. Greater BMI was also associated with lower antibody reactivity (for BMI $\geq$ 35 kg/m^2 versus normal weight, 9.8%; 95% CI 0.4-18.3).

Natural infection with COVID-19 was strongly associated with higher anti-S1 antibody response. Compared to un-infected participants, those with non-hospitalized COVID-19 showed 31.8% (95% CI, 16.5 -48.9) higher antibody levels and those with hospitalized COVID-19 showed even greater responses (58.1%, 95% CI 26.5-97.6). In adjusted models, post-vaccination infections were not significantly associated with antibody response, although the sample size was small, and unadjusted data suggested higher antibody responses in all participants with history of natural infection (**Figure 2**). Anti-nucleocapsid antibody level, an objective marker of natural infection, was also significantly associated with higher anti-S1.

Results were similar in complete case analyses (**eTable S1 in the Supplement**).

Risk factors for antibody waning

Based on a linear spline model, the slope of anti-S1 over time sharply increased in the initial 60 days after vaccination (**eTable S2 in the Supplement**). The anti-S1 levels increased 83.1% (95% CI 66.0-101.8) per month. After 60 days since vaccination, levels declined 23.0% (95% CI 20.9-24.9) per month.

Based on the largest effect estimates from our multivariable regression analysis, we examined continuous associations of vaccine type, age, sex, and diabetes status with anti-S1 over time (i.e., time between vaccine dose and DBS) using linear spline models and shown in **Figure 3**. Those who received the BNT162b2 (Pfizer-BioNTech) vaccine had lower anti-S1 levels compared with mRNA-1273 (Moderna) vaccine and this difference appeared to persist over time (**Figure 3A**). Similarly, men, adults over the age of 80 years, and those with a history of diabetes each had lower anti-S1 levels compared with their respective reference groups (**Figures 3B-D**).

In models testing for differences in the slope of anti-S1 waning starting at 60 days since initial vaccine dose, the monthly percentage loss of anti-S1 MFI 3.4% (95%CI 0.3 to 6.6) greater in receipients of the BNT162b2-Pfizer mRNA vaccine compared with mRNA-Moderna vaccine (**eTable S3 in the Supplement**). The rate of antibody waning also increased monotonically with age; participants over 80 years realized an anti-S1 MFI decline that was 13.8% per month (4.4 to 22.4) greater than adults less than 50 years old. Male sex, cigarette smoking, and diabetes were not significantly associated with anti-S1 rate of decline.

DISCUSSION

COVID-19 vaccination was associated with robust antibody response in a large multi-ethnic U.S.-based population cohort. Nonetheless, the data suggest that certain groups – including men, former and current smokers, those with higher BMI or a diagnosis of diabetes – mounted significantly lower antibody responses, and that older adults had both lower peak antibody responses to vaccination and faster antibody waning. A history of COVID-19 infection and receipt of the mRNA-1273 vaccine were associated with higher antibody responses.

Several of the factors associated with lower anti-S1 MFI have been shown to predict higher risk of COVID-19 infection and severity including hospitalization and death.[19-21] Our findings suggest possible overlapping processes that impair appropriate immunological responses to vaccine and the body's response to COVID-19 infection. Innate immune system dysfunction has been implicated as a risk factor for COVID-19 infection susceptibility and for driving different clinical trajectories.[22] Our results similarly suggest that adults at higher risk of severe COVID-19 infection (i.e., older, male, smokers, co-morbidities) also appear to mount a lower anti-S1 response to vaccines which might also explain their heightened vulnerability. In a prior United Kingdom study, higher levels correlated with a lower risk of future infection in a population-based cohort.[9] Therefore, lower anti-S responses to vaccines in specific populations (i.e., organ transplant recipients, adults that use immunosuppression medications, and older individuals) have justified additional vaccine doses.[23-25]

Our results confirm and extend prior studies of post-vaccination antibody response. Notably these studies used cohorts restricted to individuals with a limited number of risk factor assessments or lacking in pre-pandemic covariate data.[17] Moreover, while some studies have included non-white groups like self-reported Asian and Black, there have been fewer studies that have included two of the most severely impacted U.S. communities, Hispanic and AIAN. Our work is distinguished by its use of both pre-pandemic and prospectively collected risk factor data in racially and ethnically diverse U.S. cohorts, thereby reducing the role of confounding by pre-pandemic phenotypes and enhancing generalizability to the U.S. population. We identified similar factors associated with lower anti-S1 responses as the UK REACT-2 study, which quantified antibody reactivity up to 20 weeks after vaccination among a sample mostly receiving either BNT162b2 or ChAdOx1 vaccines. Nonetheless, our work differs in several respects, including the use of a continuous measure of antibody response and analysis of the mRNA-1273 vaccine (one of the most commonly administered COVID-19 vaccines in the U.S).[14]

Importantly, our data substantially extend the timeline of post-vaccination response compared with REACT-2 and other prior studies.[14] Consistent with prior literature, we observed that anti-S1 levels peaked at around 60 days after vaccination with a subsequent decline.[5-7] Anti-S1 peak levels were lower among

certain subgroups based on risk factors like older age, male sex, and diabetes, and these differences persisted over time. Older age was associated with a more rapid decline after the initial peak whereas the mRNA-1273 vaccine was associated with a slower decline. Collectively, our findings suggest vaccine strategies that both augment the initial anti-S1 peak levels and slow the rate of anti-S1 decline in certain at-risk groups may have public health implications. Additionally, it is possible that the timing of future boosters should differ for high-risk groups.

Our work confirms that antibody responses are higher in persons with a history of both vaccination and natural infection, or "hybrid" immunity. Among vaccinated participants, history of severe natural infection was associated with the highest antibody levels. This is mainly consistent with prior literature on antibody production following severe COVID illness, in which a higher viral load and longer infection duration may contributes to higher antibody levels.[26-28] Although, certain severely affected patients have been found to mount lower antibody responses, which has been hypothesized to contribute to impaired clearance of and recovery from SARS-CoV-2 in some individuals. We did not confirm a difference in antibody response between non-infected participants and participants who were infected following vaccination ("breakthrough" cases), although power was limited. Since we excluded participants who had received or were eligible for a third vaccine, the impact of additional doses could not be assessed. There is considerable evidence that these doses further enhance the antibody response, although whether the associations found in this report persist following subsequent vaccine doses merits further investigation.[29,30] Furthermore, the serology used in this study were completed prior to the outbreak of the Omicron variants, which demonstrate different immunogenicity compared to earlier variants. Future studies that can quantify the importance of variants in immune response following vaccination will be important.

Strengths and Limitations

Strengths of our study include a highly characterized, multi-ethnic, US general population-based sample; quantitative assessment of anti-S1 IgG responses using validated methods; and extended follow-up after vaccination. Nonetheless, there are several limitations. First, information on neutralizing antibodies

was not available. Prior work suggests that anti-S1 IgG is highly correlated with neutralization activity. Second, while a clinically-meaningful cutoff for the anti-S1 measure has not yet been validated, we employed a threshold that was developed with reference to pre-pandemic samples and found to be specific for natural infection in large surveillance programs.[16] Third, we did not have repeat DBS collections from participants to examine within-person temporal trends in anti-S1 levels. Nonetheless, our findings are similar to those from vaccine randomized controlled trials with repeated measures. Fourth, certain candidate risk factors for differential antibody response were self-reported, and other objectively measured co-morbidities were assessed several years prior to the serosurvey. Compared to most observational studies, however, these cohorts use highly standardized interviews and exams, including physiologic and biomarker measures, yielding robust measures. Time lags between the serosurvey and co-morbidity assessment would be expected to bias our results conservatively to the extent that incident disease would be misclassified. Although pooling data across studies may introduce heterogeneity, C4R leveraged extensive experience in harmonizing cross-cohort data and ascertained COVID-related exposures using standardized measures.[15] Hence, COVID-19 infection history was defined by integrating information from self-report on C4R questionnaires, hospital records, and anti-N antibody response, a biomarker of natural infection. Due in part to the effectiveness or the COVID-19 vaccines with respect to pre-Omicron variants, we did not have sufficient incident COVID-19 infections post-vaccination to test associations with anti-S1 antibody levels, although this may be possible with additional follow-up in C4R.

Conclusions

In summary, we identified differential anti-S1 antibody responses related to vaccine-related, socio-demographic, and clinical factors in a diverse U.S. population sample. Our findings might help to identify subgroups of adults who might benefit from more frequent vaccination or other COVID-19 prevention strategies. Strategies to optimize COVID-19 vaccine responsiveness at the individual level, and deployment at the population level, warrant further research and development.

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Figure Legends

Figure 1. Flow chart of participants with vaccine and dried blood spot data from the Collaborative Cohort of Cohorts for COVID-19 Research.

Figure 2. Anti-S1 MFI by COVID-19 infection history over time between vaccine and dried blood spot collection

Figure 3. Anti-S1 MFI over time between vaccine dose and dried blood spot collection by (A) BNT162b2 mRNA (Pfizer-BioNTech) or mRNA-1273 (Moderna) vaccine use, (B) age, (C) sex, and (D) diabetes history.

Table 1 Baseline characteristics

		Anti-S1 Quartiles			
Characteristic	**Overall**	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**
No. of participants	6797	1700	1699	1699	1699
Anti-S1 antibody MFI (log-transformed)	8.4 (1.4)	6.6 (1.0)	8.2 (0.3)	9.0 (0.3)	10.0 (0.3)
Natural log transformed-anti-N antibody MFI (SE)	4.7 (1.1)	4.4 (0.8)	4.5 (0.8)	4.7 (1.0)	5.3 (1.5)
Age					
Less than 50 years	261 (3.9%)	36 (2.2%)	60 (3.6%)	78 (4.7%)	87 (5.2%)
50-64 years	1365 (20.5%)	238 (14.5%)	347 (20.9%)	397 (23.7%)	383 (22.7%)
65-79 years	3359 (50.4%)	903 (54.8%)	894 (53.9%)	777 (46.3%)	785 (46.6%)
80 years and greater	1683 (25.2%)	470 (28.5%)	357 (21.5%)	425 (25.3%)	431 (25.6%)
Female sex	3891 (58.5%)	842 (51.2%)	962 (58.0%)	1031 (61.8%)	1056 (63.0%)
Self-reported race/ethnicity					
Non-Hispanic white	5132 (77.2%)	1297 (78.8%)	1254 (75.7%)	1295 (77.6%)	1286 (76.8%)
African-American or Black	1135 (17.1%)	286 (17.4%)	311 (18.8%)	268 (16.1%)	270 (16.1%)
Hispanic	59 (0.9%)	11 (0.7%)	13 (0.8%)	13 (0.8%)	22 (1.3%)
Asian	203 (3.1%)	24 (1.5%)	59 (3.6%)	70 (4.2%)	50 (3.0%)
American Indian and Alaskan Native	115 (1.7%)	27 (1.6%)	20 (1.2%)	22 (1.3%)	46 (2.7%)
Education attainment					
Less than high school	252 (3.9%)	63 (3.9%)	61 (3.8%)	57 (3.5%)	71 (4.4%)
High school	1392 (21.5%)	353 (22.0%)	318 (19.6%)	336 (20.6%)	385 (23.7%)
College	1253 (19.3%)	338 (21.0%)	370 (22.8%)	309 (19.0%)	236 (14.5%)
Beyond college	3586 (55.3%)	854 (53.1%)	875 (53.9%)	927 (56.9%)	930 (57.3%)
Study cohort					
ARIC	1813 (26.7%)	286 (16.8%)	308 (18.1%)	551 (32.4%)	668 (39.3%)
CARDIA	148 (2.2%)	37 (2.2%)	51 (3.0%)	34 (2.0%)	26 (1.5%)
COPDGene	457 (6.7%)	150 (8.8%)	137 (8.1%)	94 (5.5%)	76 (4.5%)
FHS	1480 (21.8%)	185 (10.9%)	316 (18.6%)	415 (24.4%)	564 (33.2%)
JHS	36 (0.5%)	8 (0.5%)	7 (0.4%)	13 (0.8%)	8 (0.5%)
MASALA	158 (2.3%)	13 (0.8%)	48 (2.8%)	62 (3.6%)	35 (2.1%)
MESA	231 (3.4%)	80 (4.7%)	71 (4.2%)	53 (3.1%)	27 (1.6%)
PrePF	75 (1.1%)	24 (1.4%)	19 (1.1%)	17 (1.0%)	15 (0.9%)
REGARDS	2147 (31.6%)	848 (49.9%)	682 (40.1%)	408 (24.0%)	209 (12.3%)
SARP	51 (0.8%)	14 (0.8%)	15 (0.9%)	8 (0.5%)	14 (0.8%)
SHS	105 (1.5%)	26 (1.5%)	18 (1.1%)	20 (1.2%)	41 (2.4%)
SPIROMICS	96 (1.4%)	29 (1.7%)	27 (1.6%)	24 (1.4%)	16 (0.9%)
Smoking status					
Never	3053 (46.0%)	680 (41.3%)	755 (45.5%)	816 (49.0%)	802 (47.9%)
Former	2980 (44.9%)	791 (48.1%)	732 (44.1%)	716 (43.0%)	741 (44.3%)
Current	609 (9.2%)	175 (10.6%)	171 (10.3%)	132 (7.9%)	131 (7.8%)
Body mass index, kg/m^2					
<25 kg/m^2	1701 (25.9%)	419 (25.8%)	418 (25.5%)	424 (25.7%)	440 (26.5%)
25-29.9 kg/m^2	2567 (39.0%)	609 (37.5%)	640 (39.0%)	670 (40.6%)	648 (39.0%)

30-34.9 kg/m^2	1437 (21.9%)	353 (21.7%)	376 (22.9%)	341 (20.7%)	367 (22.1%)
>35 kg/m^2	869 (13.2%)	244 (15.0%)	205 (12.5%)	214 (13.0%)	206 (12.4%)
Hypertension	3602 (54.5%)	1002 (61.2%)	887 (53.8%)	839 (50.6%)	874 (52.4%)
Diabetes	1233 (18.7%)	398 (24.3%)	299 (18.2%)	257 (15.5%)	279 (16.8%)
Cardiovascular disease	365 (8.8%)	81 (10.7%)	85 (9.6%)	88 (7.6%)	111 (8.2%)
Chronic obstructive pulmonary disease	431 (10.7%)	117 (16.4%)	100 (11.9%)	110 (9.8%)	104 (7.6%)
Asthma	484 (12.4%)	113 (17.0%)	103 (13.0%)	112 (10.2%)	156 (11.6%)
Chronic kidney disease	198 (3.2%)	60 (3.9%)	49 (3.2%)	54 (3.5%)	35 (2.3%)
COVID-19 infection prior to DBS collection					
No infection	3668 (82.5%)	741 (91.5%)	842 (89.9%)	1037 (86.2%)	1048 (70.1%)
Infection prior to vaccine-not severe	439 (9.9%)	39 (4.8%)	60 (6.4%)	102 (8.5%)	238 (15.9%)
Infection prior to vaccine-severe	105 (2.4%)	10 (1.2%)	3 (0.3%)	19 (1.6%)	73 (4.9%)
Infection after vaccine-not severe	234 (5.3%)	20 (2.5%)	32 (3.4%)	45 (3.7%)	137 (9.2%)
SARS-CoV-2 vaccine type					
Vaccine dose number					
1 dose	617 (9.1%)	118 (6.9%)	96 (5.7%)	155 (9.1%)	248 (14.6%)
2 doses	6180 (90.9%)	1582 (93.1%)	1603 (94.3%)	1544 (90.9%)	1451 (85.4%)
Vaccine type					
BNT162b2 mRNA (Pfizer-BioNTech)	3408 (50.1%)	1142 (67.2%)	869 (51.1%)	750 (44.1%)	647 (38.1%)
mRNA-1273 (Moderna)	3278 (48.2%)	526 (30.9%)	812 (47.8%)	923 (54.3%)	1017 (59.9%)
Other (Novavax/AstraZeneca)	111 (1.6%)	32 (1.9%)	18 (1.1%)	26 (1.5%)	35 (2.1%)
Time between vaccination and DBS collection, months	3.9 (1.8)	4.7 (1.8)	4.5 (1.6)	3.6 (1.7)	2.7 (1.5)

Abbreviations: ARIC=Atherosclerosis Risk in Communities Study; CARDIA=Coronary Artery Risk Development in Young Adults, COPDGene=Genetic Epidemiology of Chronic Obstructive Pulmonary Disease, FHS=Framingham Heart Study; JHS=Jackson Heart Study; MASALA=Mediators of Atherosclerosis in South Asians Living in America; MESA=Multi-Ethnic Study of Atherosclerosis; MFI=mean fluorescence intensity; PrePF=Preclinical Pulmonary Fibrosis; REGARDS=Reasons for Geographic and Racial Differences in Stroke; SHS=Strong Heart Study

Continuous variables presented as mean (standard deviation)
Categorical variables presented as number of participants (percentage)
Chronic kidney disease defined as estimated glomerular filtration rate below 45 mL/min/1.73m^2

Table 2 Proportion of C4R participants with anti-S1 reactivity

	Time between vaccine dose and DBS				
	0-30 days	31-60 days	61-120 days	121-180 days	181-270 days
Overall	273 (88.6%)	998 (96.4%)	2085 (98.6%)	2236 (97.5%)	1004 (96.0%)
Vaccine type					
BNT162b2 (Pfizer)	143 (86.7%)	508 (95.5%)	1027 (98.2%)	1076 (96.8%)	519 (93.9%)
mRNA-1273 (Moderna)	126 (91.3%)	470 (97.5%)	1014 (99.2%)	1133 (98.2%)	474 (98.3%)
Other	4 (80.0%)	20 (95.2%)	44 (95.7%)	27 (96.4%)	11 (100%)
Age group					
Less than 50 years	36 (81.8%)	28 (100%)	93 (98.9%)	81 (97.6%)	12 (100%)
50-64 years	99 (89.2%)	167 (98.2%)	411 (99%)	471 (99.2%)	185 (95.4%)
65-79 years	88 (89.8%)	395 (96.6%)	946 (98.9%)	1256 (97.6%)	591 (97.2%)
80 years and greater	50 (92.6%)	405 (95.3%)	624 (98.0%)	363 (95.0%)	174 (94.1%)

Abbreviations: DBS=dried blood spot

Table 3 Clinical factors associated with anti-S1 MFI after COVID-19 vaccination

Clinical risk factor	Estimated mean percent difference in anti-S1 MFI (95% confidence interval)	P-value
Age		
Less than 50 years	0.0 (ref)	
50-64 years	12.3 (- 3.63 to 30.9)	0.14
65-79 years	- 7.8 (- 20.7 to 7.3)	0.29
80 years and greater	- 30.4 (- 40.6 to - 18.5)	<0.001
Sex		
Female	0.0 (ref)	
Male	- 20.4 (- 24.8 to - 15.7)	<0.001
Race/ethnicity		
Non-Hispanic white	0.0 (ref)	
American Indian or Alaskan Native	- 3.0 (- 22.9 to 22.1)	0.79
Asian	106.3 (71.8 to 147.7)	<0.001
Black	5.3 (- 2.7 to 14.0)	0.20
Hispanic	26.5 (- 6.8 to 71.6)	0.13
Education attainment		
College or beyond	0.0 (ref)	
Less than high school	- 9.5 (- 22.0 to 5.0)	0.19
High school	- 4.6 (- 11.3 to 2.6)	0.21
Some college	- 6.2 (- 13.1 to 1.2)	0.10
Smoking history		
Never	0.0 (ref)	
Former	- 6.7 (- 12.2 to - 0.9)	0.03
Current	- 15.2 (- 23.7 to - 5.9)	0.002
Body mass index		
<25 kg/m^2	0.0 (ref)	
25-29.9 kg/m^2	6.7 (- 0.5 to 14.5)	0.07
30-34.9 kg/m^2	5.5 (- 2.8 to 14.5)	0.20
>35 kg/m^2	- 9.8 (- 18.3 to - 0.4)	0.04
Diabetes		
No	0.0 (ref)	
Yes	- 15.3 (- 21.4 to - 8.8)	<0.001
Hypertension		
No	0.0 (ref)	
Yes	- 3.1 (- 8.6 to 2.7)	0.29
Cardiovascular disease		
No	0.0 (ref)	
Yes	- 4.3 (- 14.0 to 6.5)	0.42
Chronic kidney disease		
No	0.0 (ref)	
Yes	- 7.3 (- 21.1 to 9.0)	0.14
Asthma		
No	0.0 (ref)	
Yes	- 5.0 (- 13.3 to 4.2)	0.28
Chronic obstructive pulmonary disease		
No	0.0 (ref)	
Yes	- 8.5 (- 18.6 to 2.7)	0.10
Log-transformed anti-N MFI (per 1-unit increment)	39.7 (35.3 to 44.3)	<0.001
COVID-19 infection prior to DBS collection		
No infection	0.0 (ref)	
Infection prior to vaccine-not severe	31.8 (16.5 to 48.9)	<0.001
Infection prior to vaccine-severe	58.1 (26.5 to 97.6)	<0.001
Infection after vaccine	- 0.6 (- 17.2 to 19.5)	0.95
COVID-19 vaccine type		
BNT162b2 mRNA (Pfizer-BioNTech)	0.0 (ref)	
mRNA-1273 (Moderna)	94.4 (84.2 to 105.4)	<0.001
Other (Johnson & Johnson/AstraZeneca)	3.6 (- 16.5 to 28.4)	0.75

Time between vaccine and DBS collection (per 30 days)	- 15.0 (- 17.1 to - 13.1)	<0.001
COVID-19 vaccine doses		
Two doses	0.0 (ref)	
One dose	- 39.0 (- 45.5 to - 31.9)	<0.001

Abbreviations: MFI=mean fluorescence intensity

Chronic kidney disease defined as estimated glomerular filtration rate below 45 mL/min/1.73m

Table S1 Sensitivity analysis using C4R cohort with complete covariate data

Clinical risk factor	Estimated mean percent difference in anti-S1 MFI (95% confidence interval)	P-value
Age		
Less than 50 years	0.0 (ref)	
50-64 years	10.4 (-10.1 to 35.8)	0.35
65-79 years	-1.1 (-19.6 to 21.8)	0.92
80 years and greater	-22.4 (-37.4 to -3.8)	0.02
Sex		
Female	0.0 (ref)	
Male	-17.1 (-24.5 to -9.1)	<0.001
Race/ethnicity		
Non-Hispanic white	0.0 (ref)	
American Indian or Alaskan Native	16.1 (-20.4 to 69.0)	0.44
Asian	-1.5 (-57.3 to 127.3)	0.97
Black	9.5 (-6.2 to 27.9)	0.25
Hispanic	-3.1 (-45.7 to 72.6)	0.91
Education attainment		
College or beyond	0.0 (ref)	
Less than high school	-17.6 (-35.1 to 4.7)	0.11
High school	-10.7 (-20.4 to 0.3)	0.06
Some college	-18.9 (-29.0 to -7.5)	0.002
Smoking history		
Never	0.0 (ref)	
Former	-1.7 (-10.5 to 8.0)	0.72
Current	-7.2 (-22.7 to 11.4)	0.42
Body mass index		
<25 kg/m^2	0.0 (ref)	
25-29.9 kg/m^2	11.5 (-0.1 to 24.6)	0.05
30-34.9 kg/m^2	2.1 (-10.5 to 16.5)	0.76
>35 kg/m^2	-5.8 (-19.9 to 10.7)	0.47
Diabetes		
No	0.0 (ref)	
Yes	-16.8 (-26.5 to -5.9)	0.003
Hypertension		
No	0.0 (ref)	
Yes	2.8 (-6.4 to 12.9)	0.56
Cardiovascular disease		
No	0.0 (ref)	
Yes	-11.3 (-24.6 to 4.4)	0.15
Chronic kidney disease		
No	0.0 (ref)	
Yes	-26.8 (-43.9 to -4.4)	0.02
Asthma		
No	0.0 (ref)	
Yes	-6.4 (-19.0 to 8.1)	0.37
Chronic obstructive pulmonary disease		
No	0.0 (ref)	
Yes	-14.1 (-28.4 to 3.0)	0.10
Log-transformed anti-N MFI (per 1-unit increment)	28.5 (22.0 to 35.4)	<0.001
COVID-19 infection prior to DBS collection		
No infection	0.0 (ref)	
Infection prior to vaccine-not severe	53.0 (22.5 to 91.0)	<0.001
Infection prior to vaccine-severe	106.9 (47.3 to 190.4)	<0.001
Infection after vaccine	1.2 (-24.7 to 35.9)	0.94
COVID-19 vaccine type		
BNT162b2 mRNA (Pfizer-BioNTech)	0.0 (ref)	
mRNA-1273 (Moderna)	82.9 (67.4 to 99.8)	<0.001

Other (Johnson & Johnson/AstraZeneca)	-4.4 (-29.5 to 29.6)	0.77
Time between vaccine and DBS collection (per 30 days)	-5.7 (-9.9 to -1.5)	0.009
COVID-19 vaccine doses		
Two doses	0.0 (ref)	
One dose	-45.0 (-52.8 to -36.0)	<0.001

Abbreviations: MFI=mean fluorescence intensity

Chronic kidney disease defined as estimated glomerular filtration rate below 45 mL/min/1.73m

Table S2 Change in anti-S1 before and after 60 days from vaccination

Risk factors	Mean % change in anti-S1 MFI rate of change per 30 days (95% CI)
Less than 60 days from vaccination	83.1 (66.0 to 101.8)
Greater than 60 days from vaccination	-23.0 (-24.9 to -20.9)

Abbreviations: CI=confidence interval; MFI=mean fluorescence intensity

Linear spline model adjusted for age, sex, race/ethnicity, smoking history, education attainment, body mass index, diabetes, cardiovascular disease, asthma, chronic obstructive pulmonary disease, anti-N, COVID-19 infection history, dried blood spot batch, vaccine type, and vaccine dose.

Table S3 Associations of risk factors with changes in anti-S1 MFI 60 days after vaccination

Risk factors	Mean % change in anti-S1 MFI rate of change per 30 days (95% CI)
Male (ref. female)	-1.5 (-4.7 to 1.8)
Age	
50-64 years (ref. less than 50 years)	-11.0 (-19.7 to -1.2)
65-79 years (ref. less than 50 years)	-10.5 (-19.1 to -0.9)
80 years and greater (ref. less than 50 years)	-13.8 (-22.4 to -4.4)
Smoking	
Former (ref. never)	-1.7 (-5.0 to 1.7)
Current (ref. never)	-3.6 (-9.2 to 2.4)
Diabetes (ref. no diabetes)	3.9 (-0.4 to 8.3)
Vaccine type	
BNT162b2 mRNA- Pfizer-BioNTech (ref. mRNA-1273-Moderna)	-3.4 (-6.6 to -0.3)
Other (Johnson & Johnson/AstraZeneca) (ref. mRNA-1273-Moderna)	9.4 (-4.7 to 25.5)

Abbreviations: CI=confidence interval; MFI=mean fluorescence intensity

Linear spline model adjusted for age, sex, race/ethnicity, smoking history, education attainment, body mass index, diabetes, cardiovascular disease, asthma, chronic obstructive pulmonary disease, anti-N, COVID-19 infection history, dried blood spot batch, vaccine type, and vaccine dose.

Figure 1.

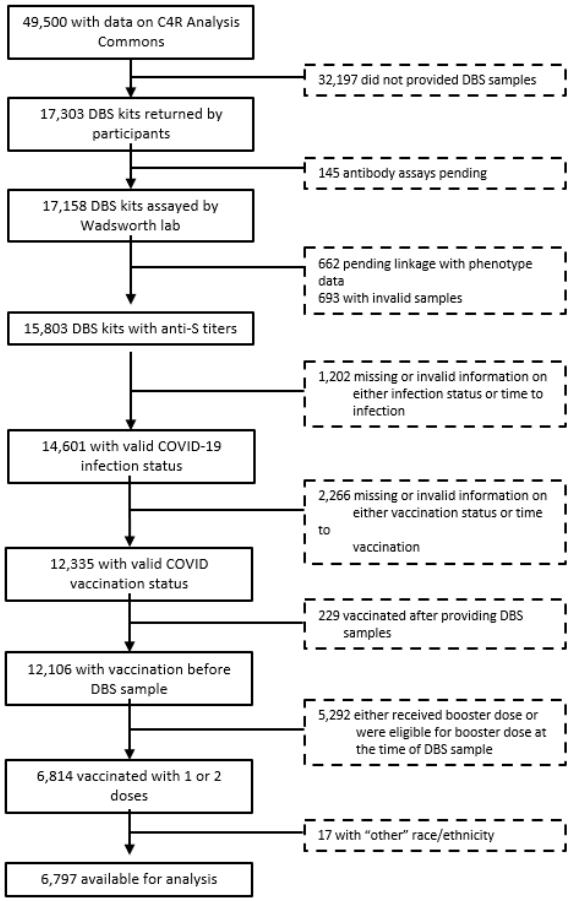
49,500 with data on C4R Analysis Commons
32,197 did not provided DBS samples
17,303 DBS kits returned by participants
145 antibody assays pending
17,158 DBS kits assayed by Wadsworth lab
662 pending linkage with phenotype data
693 with invalid samples
15,803 DBS kits with anti-S titers
1,202 missing or invalid information on either infection status or time to infection
14,601 with valid COVID-19 infection status
2,266 missing or invalid information on either vaccination status or time to vaccination
12,335 with valid COVID vaccination status
229 vaccinated after providing DBS samples
12,106 with vaccination before DBS sample
5,292 either received booster dose or were eligible for booster dose at the time of DBS sample
6,814 vaccinated with 1 or 2 doses
17 with "other" race/ethnicity
6,797 available for analysis

Figure 2. Anti-S1 by prior COVID-19 infection history

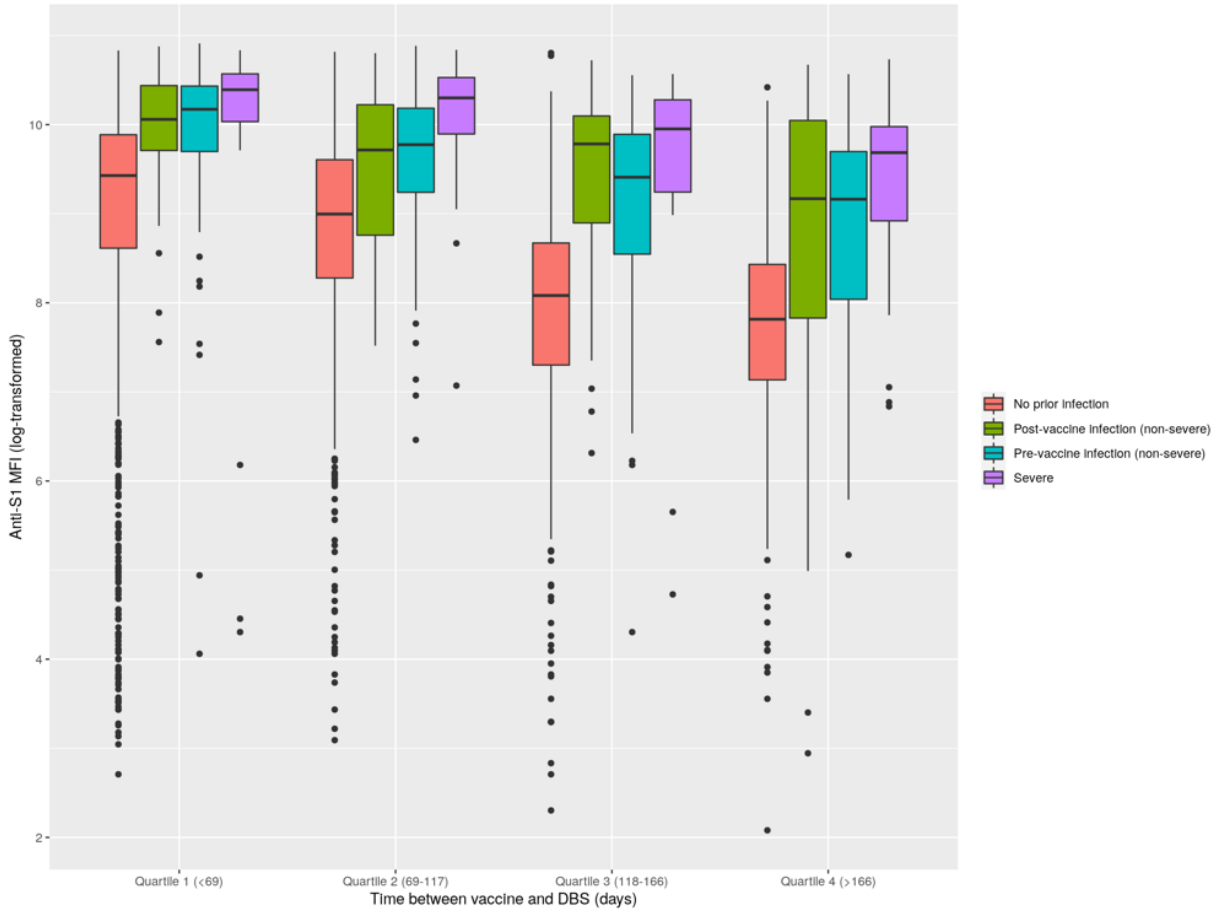

Figure 3A. Anti-S1 over time overall

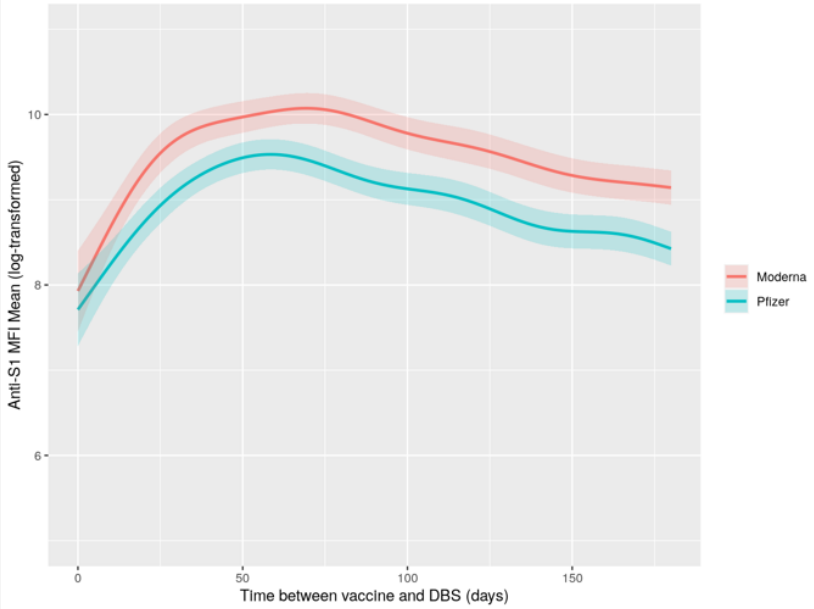

Figure 3B. Age

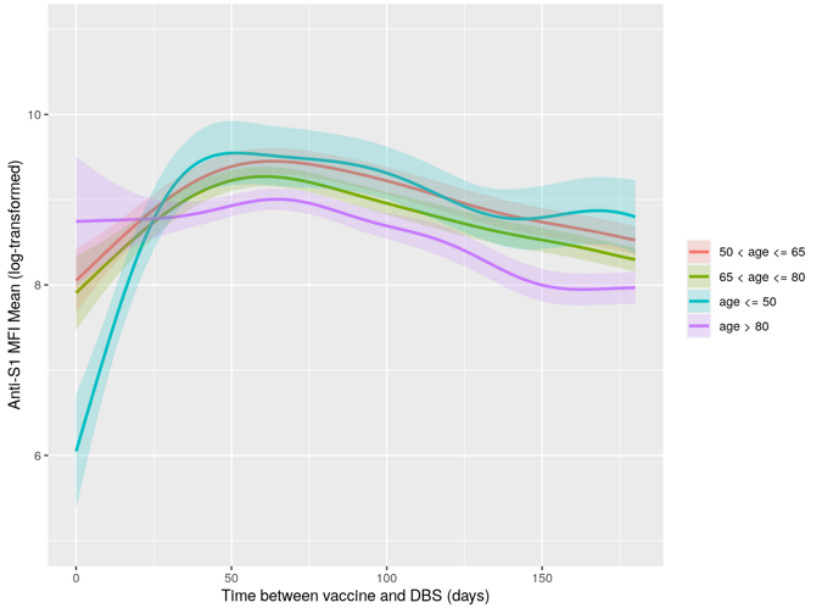

Figure 3C. Sex

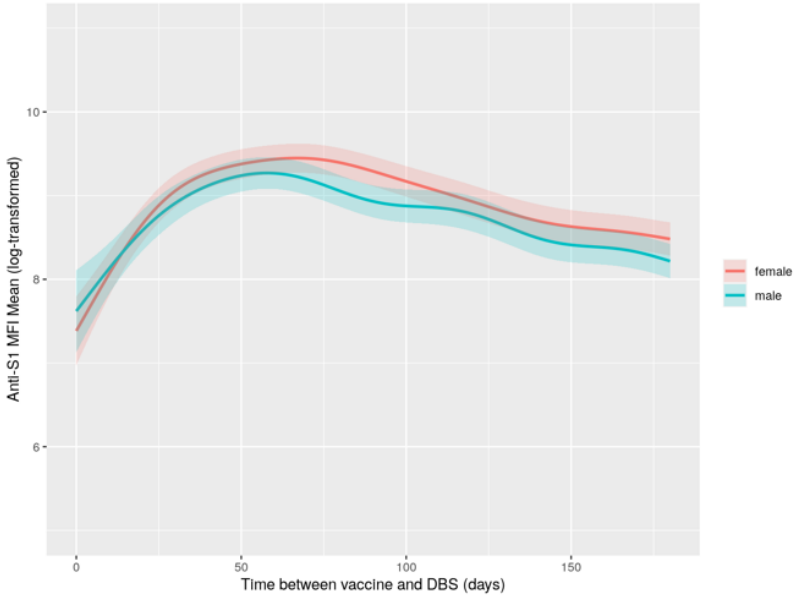

Figure 3D. Diabetes