Demographic and clinical factors associated with SARS-CoV-2 Anti-Nucleocapsid Antibody Response Among Previously Infected US Adults: The C4R Study

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ABSTRACT

Despite the availability of effective vaccines and a recent decrease in annual deaths, coronavirus disease-2019 (COVID-19) remains a leading cause of death. Serological studies provide insights into host immunobiology of adaptive immune response to infection which holds promise for identifying high-risk individuals for adverse COVID-19 outcomes. We investigated correlates of anti-nucleocapsid (N) antibody responses following Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection in a U.S. population-based meta-cohort of adults participating in longstanding NIH-funded cohort studies. Anti-nucleocapsid antibodies were measured from dried blood spots collected between February 2021-February 2023. Among 2,152 Collaborative Cohort of Cohorts for COVID-19 Research (C4R) participants with prior SARS-CoV-2 infection, the mean age (standard deviation) was 62.7(12.5), 63% were women, and 62% self-reported membership in a race/ethnicity minority group. The proportion of participants reactive to anti-nucleocapsid peaked at 56% by 6 months post-infection and waned to only 37% ≥12 months after infection. Higher anti-nucleocapsid antibody response was associated with older age, male sex, lower income, and former smoking. Hispanic ethnicity and vaccination (even after infection) were associated with lower anti-nucleocapsid reactivity. Hospitalization with critical COVID-19 was marginally associated with increased response while common cardiometabolic co-morbidities were not associated with anti-nucleocapsid response. These findings inform the underlying immunobiology of adaptive immune response to infection, as well as the potential utility of anti-nucleocapsid antibody response for clinical practice and COVID-19 serosurveillance.

INTRODUCTION

The persistence of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) variants poses an ongoing challenge to public health even as the virus transitions to endemic status. Coronavirus disease-2019 (COVID-19) remains a top 10 leading cause of death as of 2023, and post-COVID-19 conditions affect a considerable proportion of survivors[1]. Identifying individuals and populations at high-risk for adverse COVID-19 outcomes is a high priority.

Serological studies provide insights into host immunobiology of adaptive immune response to infection. This knowledge holds promise for identifying high-risk individuals for adverse COVID-19 outcomes, including acute and post-acute conditions, and the development of prevention and treatment strategies. Although prior work has examined correlates of the anti-spike (S) antibody response,[2-6] correlates of the anti-nucleocapsid (N) antibody response—which is only generated by natural infection, not by vaccination—are relatively understudied. Milder acute infection severity and history of COVID-19 vaccination have been linked to lower anti-N responses in some studies, yet results have been mixed[7-9]. Prior studies also had notable limitations including small sample sizes[7,10], limited representation of ages >65 years[7-9,11], lack of precise information on timing of infection[8,9], inability to account for potentially important risk factors (e.g., smoking status, adiposity, clinical conditions), and study completion prior to the emergence of the less virulent Omicron variant.

In addition, anti-N serum IgG antibodies are used to classify history of SARS-CoV-2 infection in clinical practice, surveillance, and research. Differential anti-nucleocapsid antibody response may lead to differential misclassification and inclusion in studies of post-acute sequelae of SARS-CoV-2 infection (PASC). Hence, understanding the correlates of the anti-N response may inform strategies to reduce misclassification and selection bias in clinical and research settings.

We investigated the correlates of the anti-nucleocapsid antibody response following SARS-CoV-2 infection in a large, diverse, US population-based meta-cohort of adults. We assessed the dynamics of the antibody response over time-since-infection. Then, accounting for temporal trends, we examined the association of anti-nucleocapsid response with socio-demographics, pre-pandemic health and lifestyle factors, acute illness severity, and COVID-19 vaccination history.

METHODS

Participants

The Collaborative Cohort of Cohorts for COVID-19 Research (C4R) is performing standardized assessments of COVID-19 in participants in 14 longstanding NIH-funded prospective cohort studies (see **Supplemental Methods**)[12] to study the impact of the COVID-19 pandemic on US adults. Cohort participants who were alive on March 1, 2020, and had not withdrawn consent for cohort participation, were considered eligible for C4R enrollment. Institutional review board approval was obtained from all study sites. Informed consent was obtained from each study participant.

Inclusion criteria for the present analysis were prior SARS-CoV-2 infection, assessed via self-report and/or review of hospital discharge records, prior to submission of a valid dried blood spot (DBS) or a venous serum sample for serology assay (Supplemental Figure 1).

Serosurvey

C4R serological assays were performed on DBS or stored serum, as previously described.[12,13] Briefly, the DBS requires that several drops of whole blood from a finger prick or blood collection tube be absorbed into a bar-coded, Whatmann filter paper card. Participants who consented to the serosurvey completed the DBS at home or at an in-person exam. DBS samples were shipped via United States mail to the University of Vermont. The present study includes DBS or serum samples collected between February 2021 and February 2023.

Serological assays were performed on DBS eluates or serum by the Wadsworth Center, New York State Department of Health (Albany, NY, USA), using validated methods.[13] 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, and nucleocapsid (N) protein, which is induced by natural infection only. Briefly, SARS-CoV-2 S1 and N antigens (Sino Biological,

Wayne, PA, USA) were covalently coupled to 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). Median 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-nucleocapsid MFI values in uninfected (pre-pandemic) DBS samples.[13] For each bead set, the reactivity threshold was calculated as the mean +6 standard deviations of the uninfected (pre-pandemic) MFI. Samples above this threshold were classified as reactive.

Infection and vaccination history

C4R collected information on SARS-CoV-2 infection and vaccination status via two waves of questionnaires conducted from April 2020 through February 2023 as previously reported[14]; the current report includes data from questionnaires collected through February 2023. Questionnaires were administered by telephone interview, electronic survey, in-person examination, and/or mailed pamphlet. Participants were asked about history of SARS-CoV-2 infection, SARS-CoV-2 testing, COVID-19 hospitalization, COVID-19 vaccination status, date of first vaccine administration, number of vaccines received, and vaccine manufacturer. Infections were dichotomized according to whether they were or were not associated with hospitalization. Confirmed history of SARS-CoV-2 infection was classified based on self-report of a positive SARS-CoV-2 test or adjudication of medical records for a COVID-19 hospitalization; self-reported infections not meeting either of these criteria were classified as probable. Both confirmed and probable cases of infection were included in the main analyses, and a sensitivity analysis was performed that excluded probable cases.

Pre-pandemic Measures

C4R cohorts have performed longitudinal data collection on participants for up to 51 years. C4R harmonized these data across cohorts, as previously described[12]. Age, sex, and educational attainment were self-reported. Race and ethnicity, which were self-reported and categorized according to the 2000 Census methods[15,16], were included in this study to address specific knowledge gaps about disparities in COVID-19 outcomes among members of historically marginalized or underserved populations. Time-varying risk factors were defined using the most recent data collected by each cohort. Smoking status was self-reported as never, former, or current. Height, weight, blood pressure, fasting lipids, and blood glucose were measured using standardized protocols. Body mass index (BMI) was calculated as weigh in kilograms divided by height in meters and classified into standard CDC-defined weight categories. Hypertension was defined as a systolic blood pressure≥140 mm Hg, diastolic blood pressure≥90 mm Hg, or antihypertensive medication use. Diabetes was defined as fasting blood glucose≥126 mg/dL or use of diabetes medications. Cardiovascular disease, asthma, and chronic obstructive pulmonary disease (COPD) were identified via self-report or by the ascertainment of relevant clinical events, confirmed by medical record review, over cohort follow-up.

Statistical Analysis

Participant characteristics were described as mean±standard deviation (SD) for the full sample and mean±standard error (SE) among subgroups for continuous variables while categorical variables are reported as n(%). Continuous variables with evidence of non-normality were natural log-transformed prior to analysis.

The primary analysis used Poisson models to regress the probability of anti-N reactivity on candidate risk factors, time-since-infection (defined as days from infection to serosurvey),

and laboratory batch. Secondary analyses regressed continuous natural log-transformed anti-nucleocapsid IgG levels on the aforementioned variables using generalized linear models. For linear regressions estimating continuous anti-nucleocapsid MFI levels, we exponentiated the beta-coefficient and presented the results as mean percent difference in anti-nucleocapid level in the comparison vs. reference group.

Sub-group analyses were conducted to evaluate associations of severity of clinical infection with aspects of vaccination history, including timing of vaccination (before vs after infection) and manufacturer.

Multiple imputation by chained equations was applied to handle missing covariable data (Supplemental Table 1). Ten imputed datasets were created, and Rubin's rule was applied to combine the estimates from the imputed datasets.[17] Characteristics of complete cases were similar to the imputed (primary) dataset (Supplemental Table 2**)**.

All analyses were conducted in R (R Statistical Foundation, Vienna, Austria)[12]. R packages used for analysis include: tidyverse, arsenal, mice and lmtest. Two-sided p-values<0.05 were considered statistically significant.

RESULTS

Participant characteristics

Among 2,154 C4R participants with evidence of prior SARS-CoV-2 infection, the mean age (SD) was 62.71(12.5) years, 63% were women, and 38% self-reported race/ethnicity as white non-Hispanic (Table 1). Chronic disease prevalence and/or cardiometabolic risk factor levels were high: >80% of this sample was overweight or obese, 53% had hypertension and 24% had diabetes. Participants with nucleocapsid antibody reactivity tended to be older, men, former smokers, underweight or obese. Higher anti-nucleocapsid MFI levels were also associated with a history of COVID-19 hospitalization, being unvaccinated at the time of serosurvey, and being between 1 month and 6 months from infection (Table 1 and Supplemental Table 3).

Nucleocapsid reactivity and time-since-infection

Overall, 43.4% of participants were classified as reactive to nucleocapsid. Nucleocapsid reactivity was highest in the 1-6 month post-infection period, plateuaued, and declined thereafter (Table 2 & Supplemental Table 4). Among participants with self-reported infection 12 or more months prior to the serosurvey, only 37.1% were classified as nucleocapsid-reactive.

Correlates of nucleocapsid reactivity

After multivariable adjustment, increased probability of nucleocapsid reactivity was associated with older age (≥80 vs <50 years), former smoking, higher anti-S1 MFI, lower income, being unvaccinated at time of serosurvey, and a time interval of <6 months between infection and DBS; history of COVID-19 hospitalization with critical illness was marginally associated with an increased probability of reactivity (Table 3, Figure 1A). Hispanic ethnicity was associated with a reduced probability of reactivity (Table 3, Figure 1A). Multivariable adjustment had minimal influence on the results. Results were generally consistent in

multivariable linear regressions assessing correlates of anti-nucleocapsid MFI levels although men and individuals reporting American Indian or Alaskan Native race had statistically significantly higher nucleocapsid MFI levels (Figure 1B, Supplemental Table 5).

Acute illness severity

Among hospitalized participants (vs. not hospitalized), nucleocapsid reactivity prevalence was lower in the first 3 months following infection but higher three months and beyond (p-value for interaction between hospitalized COVID-19 and time since infection = 0.45, Figure 2A).

Association between vaccination history and nucleocapsid-reactive antibody titers

Vaccination was associated with reduced serum IgG nucleocapsid-reactivity, whether or not individuals were vaccinated before (RR=0.37;95%CI:0.30,0.46) or after (RR=0.51;95%CI:0.45,0.58) infection. This observation remained after multivariable adjustment that included time since infection. Of note, time between infection and serosurvey differed among participants who were unvaccinated (291.91±162.91 days), vaccinated after infection (395.01±167.70 days), or vaccinated before infection (138.86±113.69), (p-value=0.05, Supplemental Table 7 and Supplemental Figure 2). While vaccination before or after infection was associated with a notably lower prevalence of reactivity overall, the lower prevalence was driven by results during the 1 – 6-month post-infection period, and prevalence differences according to vaccination status disappeared 6 months after infection as reactivity rates declined substantially in the unvaccinated (Figure 2B, p-value for interaction=0.05). There was not meaningful heterogeneity in correlates of nucleocapsid reactivity by vaccination status (Supplemental Table 6). Participant characteristics according to vaccination status are shown in

Supplemental Table 7 and the distribution of time between infection and vaccination is shown in Supplemental Figure 3.

With respect to vaccine manufacturer, compared to unvaccinated participants, nucleocapsid-reactivity was reduced among individuals receiving either the Pfizer (RR=0.81;95%CI:0.71,0.94) or Moderna (RR=0.81;95%CI:0.71,0.94) vaccines. While vaccination with the Johnson & Johnson vaccine was also associated with lower probability of reactivity (RR=0.86;95%CI:0.65,1.13), the finding was attenuated and not statistically significant. Both Novavax (RR=1.27;95%CI:0.85,1.89) and AstraZeneca (RR=1.60;95%CI:0.94,2.71) vaccines were associated with a nominally increased probability of reactivity.

DISCUSSION

In a large, diverse, population-based cohort of U.S. adults with priror SARS-CoV-2 infection, we observed that nucleocapsid reactivity peaked at 1-6 months before waning substantially, with approximately two-thirds of participants infected ≥12 months prior to the serosurvey classified as non-reactive. Higher nucleocapsid antibody reactivity was associated with several factors linked to a greater risk of severe COVID-19, including older age, male sex, former smoking, hospitalization with critical COVID-19 illness, and lack of COVID-19 vaccination. In light of these results, the question of whether the time-dependent anti-nucleocapsid antibody response may offer information on the severity of SARS-CoV-2 viremia warrants further study.

The associations of higher anti-nucleocapsid response with age, and male sex agree with a previous study among a predominantly non-Hispanic white and generally healthy sample of >19,000 blood donors[9]. The present findings extend those results by including an older, more

diverse cohort with a substantial prevalence of co-morbidities and broader assessments of socio-economic factors. Accordingly, we additionally observed that lower socio-economic status (defined by education and income) and hospitalization were related to higher anti-nucleocapsid levels.

To our knowledge, no prior study has compared anti-nucleocapsid antibody response among individuals with vs. without hospitalization. Our current findings show that hospitalization was marginally related to higher nucleocapsid reactivity prevalence, which could be explained by greater viral exposure for prolonged time periods. Interestingly, there was a trend suggesting that anti-nucleocapsid response was delayed in those hospitalized. However, this should be cautiously interpreted as there were few individuals with serologies shortly after hospitalization and the delayed response could also be explained by anti-COVID therapies administered in the hospital (e.g., corticosteroids). Future studies that measure anti-nucleocapsid trajectories longitudinally following infection will be important for confirming this finding.

The majority of factors associated with anti-nucleocapsid response following infection were inverse to our findings with respect to the anti-S1 response following vaccination. We previously reported that older age, male sex, current smoking, higher BMI, non-mRNA-based vaccines, and certain comorbidities were associate with lower anti-S1 antibody levels after receiving COVID-19 vaccinations[2]. In contrast, several comorbidities were not related to anti-nucleocapsid response, while older age, male sex and former smoking were related to higher anti-nucleocapsid response and mRNA-based vaccines were related to lower anti-nucleocapsid response. We speculate that these seemingly opposing results can be reconciled as follows. Anti-S1 response to vaccination corresponds to the host immune response to a fixed S1 exposure, providing an index of host resistance to SARS-CoV-2. The anti-nucleocapsid response to a real-world viral exposure, however, may correlate with the severity and duration of viremia, which may be inverse to host resistance. These hypotheses merit further investigation.

Another important finding in our current study was the observation that vaccination was related to lower anti-nucleocapsid response *regardless of timing vis-à-vis infection*. This relationship was not observed in prior studies[7-9]. Knowledge about timing of infection provides C4R with a unique advantage when considering the role of vaccination in antibody response following infection, as prior studies reporting on this association did not consider infection timing[8,9]. Additionally, in one prior study showing no difference in anti-nucleocapsid response between vaccinated and unvaccinated healthcare workers, the time duration between vaccination and serological testing was very short (<35 days in all participants[8]). It is possible that infection shortly after vaccination still results in greater infection severity and notable viremia as vaccine-induced immunity might still be developing, thus resulting in a greater anti-nucleocapsid antibody response. Pfizer vaccine trial results demonstrated that infection rates did not start to decrease until ~two weeks following the first vaccine dose[18], and prior serostudies report that seropositivity following vaccination is not present in the majority of participtans until 12 days post vaccination[19].

The observation that vaccination, *even after infection*, is related to lower reactivity levels (compared to the unvaccinated) is somewhat counter-intuitive as individuals unvaccinated at the time of infection may have higher viral loads and more severe infections, thus provoking a stronger antibody response. This pattern was most evident among those completing the serosurvey within 1-3 months of their infection, and as time since infection increased, reactivity prevalence estimates equalized across all groups.

There are several mechanisms by which vaccination after infection might influence antibody response. First, individuals of lower SES were less likely to be vaccinated or take other COVID-19 mitigation precautions, and more likely to be considered essential workers. Therefore, it is possible these individuals were more likely to either: i) realize higher initial infectious doses due to more social contact and less infection mitigation; and/or ii) delay seeking care due to lack of access, resulting in more severe infections. Consistent with this hypothesis,

we do see that lower SES was associated with elevated anti-nucleocapsid response in our data, but we could not account for COVID risk mitigation behaviors. Second, it is possible that unvaccinated individuals were more susceptible to unreported/unknown re-infections which 'boosted' the antibody response. Third, vaccination after infection could reduce viral persistence and chronic immune system provocation ongoing after recovery from the acute infection. Prior studies have found evidence for viral persistence for months following recovery from SARS-CoV-2 infection[20]. There is also evidence to suggest that post-infection vaccination could reduce signs and symptoms of PASC, which is consistent with idea that vaccination can reduce viral persistence and dampen the sustained immune response to infection[21,22]. Fourth, vaccination might redirect the immune system towards anti-S1 production and away from anti-nucleocapsid production suggesting a 'zero-sum immunobiology'. A recent study in mice demonstrated that in stressed mice, SARS-CoV-2 vaccination resulted in increased antibody binding affinity to the immunogen but decreased IgG levels and B cell clonal expansion[23]. Fifth, being vaccinated after infection was possibly related to infection with the Omicron variant and given the attenuated virulence of Omicron, the immune response might have been attenuated as well. Finally, while confounding related to the differential time since infection could produce spurious findings, the distribution of time since infection was very similar between the unvaccinated and those vaccinated after infection and findings remained after adjustment for time since infection.

Beyond their potential biological and clinical applications, the present findings have important implications for future research studies and clinical practice. Most notably, the fact that there are clear correlates of anti-nucleocapsid response raises the potential for differential information bias in studies of COVID-19. For example, the use of antibody results to assess prior infection in clinical practice, perhaps to rule in or out the potential for long-COVID, could lead to biased diagnostic patterns in which vaccinated individuals are less likely to receive a long-COVID diagnosis when compared to unvaccinated individuals. Additionally, while

seroprevalence studies are known to underestimate prior infection estimates, these underestimates will be more pronounced among vaccinated, younger, and more affluent populations and those with less severe prior infections.

Strengths and Limitations

C4R is a prospective, multi-ethnic, US general population-based sample that has comprehensive pre-pandemic phenotyping. Our data extends information on temporal trends as over 40% of the cohort provided DBS >1 year following infection. Additionally, C4R data provide an integrated definition of prior infection using self-report and physician-adjudicated hospital records. The use of mailed DBS kits increased our response rates, particularly among groups who were less likely to leave the home for in-person study visits during early portions of the pandemic. The use of a semi-quantitative assessment of anti-nucleocapsid IgG responses using validated methods is an important strength.

Some key limitations should be noted. First, information on neutralizing antibodies, other immunoglobulin isotypes (IgM, IgA), or measures of cellular immunity were not available. Second, a lack of repeated within person measures creates the potential for the observed time trends to be confounded by demographic and phenotypic factors. Third, the clinical significance of anti-nucleocapsid antibody response is yet to be determined. Fourth, misclassification of time-varying risk factors is possible. Clinical conditions and smoking status were measured several years prior to the serosurvey, which may conservatively bias our estimates. Infection history was defined primarily by self-report and could be subject to false-positive or false-negative classification, with uncertain influence on our estimates. Moreover, misclassification of infection could have been differential by levels of putative risk factors.

In conclusion, we observed modest levels of reactivity to an anti-nucleocapsid IgG antibody assay in a large, diverse population-based sample of U.S. adults reporting history of SARS-CoV-2 infection. Older age, lower income, former smoking, increased anti-S1 response,

hospitalization and being unvaccinated were related to higher MFI levels and reactivity prevalence. Future research that can assess longitudinal within-person patterns will be necessary to better understand predictors of antibody response trajectories and their biological underpinnings, which may include differences in severity and duration of viremia. Studies that can assess serological response to a primary infection as a predictor of future risk for severe re-infection will also help to contextualize the present findings.

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Figure Legends

Figure 1: Forest plot showing correlates of anti-nucleocapsid antibody reactivity (a) and mean florescence unit (b) among n = 2,154 C4R participants.

Figure 2: Proportion of individuals with anti-nucleocapsid antibody reactivity detected by time since infection according to severity of infection (a) and vaccination status (b) among n=2,154 C4R participants.

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TABLES

Table 1. Baseline characteristics according to IgG nucleocapsid antibody reactivity status among n= 2,154 participants in the C4R study

Characteristic	Overall	Anti-N Reactive (n=935)	Anti-N Not Reactive (n=1219)
No. of participants	2154	935	1219
Anti-S1 antibody MFI (log-transformed)	9.1(1..2)	9.3(1.0)	8.9(1.3)
Anti-N antibody MFI (log-transformed)	7.3(1.5)	8.5(0.8)	6.3(1.1)
% Reactive to Anti-S1	2122 (98.6%)	931 (99.8%)	1191 (97.7%)
% Reactive to Anti-N	933 (43.4%)	933 (100.0%)	0 (0.0%)
Age			
Less than 50 years	322 (14.9%)	121 (12.9%)	201 (16.5%)
50-64 years	949 (44.1%)	379 (40.5%)	570 (46.8%)
65-79 years	739 (34.3%)	336 (35.9%)	403 (33.1%)
80 years and greater	144 (6.7%)	99 (10.6%)	45 (3.7%)
Female sex	1349 (62.6%)	576 (61.6%)	773 (63.4%)
Self-reported race or ethnicity			
Non-Hispanic White	813 (37.7%)	398 (42.6%)	415 (34.0%)
African-American or Black	287 (13.3%)	149 (15.9%)	138 (11.3%)
Hispanic	800 (37.1%)	238 (25.5%)	562 (46.1%)
Asian	21 (1.0%)	13 (1.4%)	8 (0.7%)
American Indian and Alaskan Native	233 (10.8%)	137 (14.7%)	96 (7.9%)
Education attainment			
Less than high school	359 (16.7%)	138 (14.8%)	221 (18.1%)
High school	563 (26.1%)	263 (28.1%)	300 (24.6%)
College	475 (22.1%)	223 (23.9%)	252 (20.7%)
Beyond college	757 (35.1%)	311 (33.3%)	446 (36.6%)
Study cohort			
ARIC	72 (3.3%)	52 (5.6%)	20 (1.6%)
CARDIA	170 (7.9%)	85 (9.1%)	85 (7.0%)
COPDGene	187 (8.7%)	92 (9.8%)	95 (7.8%)
FHS	140 (6.5%)	60 (6.4%)	80 (6.6%)
HCHS/SOL	733 (34.0%)	210 (22.5%)	523 (42.9%)
JHS	26 (1.2%)	13 (1.4%)	13 (1.1%)
MASALA	12 (0.6%)	7 (0.7%)	5 (0.4%)
MESA	130 (6.0%)	58 (6.2%)	72 (5.9%)

PrePF	30 (1.4%)	14 (1.5%)	16 (1.3%)
REGARDS	345 (16.0%)	163 (17.4%)	182 (14.9%)
SARP	23 (1.1%)	16 (1.7%)	7 (0.6%)
SHS	231 (10.7%)	136 (14.5%)	95 (7.8%)
SPIROMICS	55 (2.6%)	29 (3.1%)	26 (2.1%)
Smoking status			
Never	1110 (51.5%)	435 (46.5%)	675 (55.4%)
Former	773 (35.9%)	387 (41.4%)	386 (31.7%)
Current	271 (12.6%)	113 (12.1%)	158 (13.0%)
Body mass index, kg/m^2			
<25 kg/m^2	386 (17.9%)	180 (19.3%)	206 (16.9%)
25-29.9 kg/m^2	770 (35.7%)	307 (32.8%)	463 (38.0%)
30-34.9 kg/m^2	553 (25.7%)	227 (24.3%)	326 (26.7%)
>35 kg/m^2	445 (20.7%)	221 (23.6%)	224 (18.4%)
Hypertension	1142 (53.0%)	540 (57.8%)	602 (49.4%)
Diabetes	514 (23.9%)	242 (25.9%)	272 (22.3%)
Cardiovascular disease	223 (10.4%)	103 (11.0%)	120 (9.8%)
COVID-19 infection severity			
Not hospitalized	1754 (81.4%)	738 (78.9%)	1016 (83.3%)
Non-critical hospitalization	307 (14.3%)	142 (15.2%)	165 (13.5%)
Critical hospitalization	93 (4.3%)	55 (5.9%)	38 (3.1%)
Vaccine status			
Not vaccinated	415 (19.3%)	215 (23.0%)	200 (16.4%)
Vaccinated after infection	1409 (65.4%)	586 (62.7%)	823 (67.5%)
Vaccinated before infection	330 (15.3%)	134 (14.3%)	196 (16.1%)
Time since infection and DBS collection, months	11.4(6.2)	10.7(6.3)	11.9(6.1)

Table 2. Proportion of participants with IgG nucleocapsid reactivity derived from multivariable adjusted Poisson regression models among n= 2,154 participants in the C4R study

Group	0-30 days (n=24)	31-89 days (n=149)	90-119 days (n=101)	120-180 days (n=201)	181-365 days (n=794)	>365 days (n=885)
Overall	25.0%	49.7%	56.4%	54.7%	45.1%	37.1%
Vaccine type						
Unvaccinated	20.0%	83.1%	72.9%	79.0%	46.3%	38.4%
Vaccinated after infection	n/a	61.7%	59.7%	49.0%	44.7%	36.8%
Vaccinated before infection	26.3%	39.2%	50.2%	40.5%	44.9%	36.9%
Age group						
Less than 50 years	25.0%	63.0%	48.1%	46.2%	26.3%	38.5%
50-64 years	25.0%	45.6%	60.1%	54.3%	38.4%	35.2%
65-79 years	22.2%	43.0%	44.2%	57.8%	51.0%	36.7%
80 years and greater	33.3%	71.4%	87.8%	61.2%	74.4%	58.6%
Sex						
Female	23.1%	54.5%	54.6%	56.3%	44.4%	35.2%
Male	27.3%	40.0%	59.3%	53.6%	46.2%	40.2%
Self-reported race or ethnicity						
Non-Hispanic White	20.0%	53.4%	69.8%	63.0%	50.8%	34.7%
African-American or Black	33.3%	54.5%	61.5%	70.6%	51.0%	49.5%
Hispanic	11.1%	40.0%	35.9%	41.3%	26.2%	27.8%
Asian	n/a	0.0%	0.0%	n/a	83.3%	42.9%
American Indian and Alaskan Native	100.0%	68.4%	100.0%	60.0%	53.7%	58.2%
Education attainment						
Less than high school	18.0%	40.3%	38.3%	50.8%	39.4%	38.3%
High school	50.0%	53.8%	65.0%	63.2%	47.6%	38.2%
College	35.3%	56.0%	57.8%	62.6%	46.6%	40.2%
Beyond college	5.8%	46.6%	56.6%	47.9%	44.1%	33.2%
Smoking status						
Never	28.6%	49.3%	54.5%	53.4%	39.3%	33.4%
Former	22.2%	51.9%	65.6%	56.9%	54.6%	41.6%

Current	0.0%	46.2%	39.8%	57.1%	40.6%	40.2%
Body mass index, kg/m^2						
<25 kg/m^2	2.0%	52.7%	69.1%	66.2%	48.2%	37.4%
25-29.9 kg/m^2	50.6%	51.3%	40.0%	51.1%	40.8%	34.7%
30-34.9 kg/m^2	16.5%	41.0%	64.2%	44.9%	44.4%	36.1%
>35 kg/m^2	0.0%	51.9%	78.4%	66.8%	50.5%	41.9%
Hypertension						
No	27.3%	52.1%	46.7%	51.8%	39.6%	33.5%
Yes	23.1%	47.4%	68.7%	58.5%	49.8%	40.2%
Diabetes						
No	30.1%	54.8%	57.1%	55.3%	41.7%	35.8%
Yes	7.4%	24.0%	53.8%	55.6%	55.5%	40.6%
Cardiovascular disease						
No	22.0%	48.8%	56.2%	54.8%	44.1%	37.6%
Yes	54.5%	57.5%	58.9%	59.6%	53.7%	32.6%
COPD						
No	25.0%	47.4%	58.8%	54.2%	43.9%	37.2%
Yes	n/a	65.9%	8.5%	66.2%	52.2%	35.9%
COVID-19 infection severity						
Not hospitalized	26.1%	51.1%	53.9%	54.2%	41.7%	36.5%
Non-critical hospitalization	0.0%	28.2%	72.7%	57.0%	58.9%	34.2%
Critical hospitalization	n/a	33.3%	100.0%	75.0%	67.9%	52.8%

Table 3. Clinical factors associated with risk of IgG nucleocapsid reactivity after COVID-19 infection, from multivariable adjusted Poisson regression among n= 2,154 participants in the C4R study

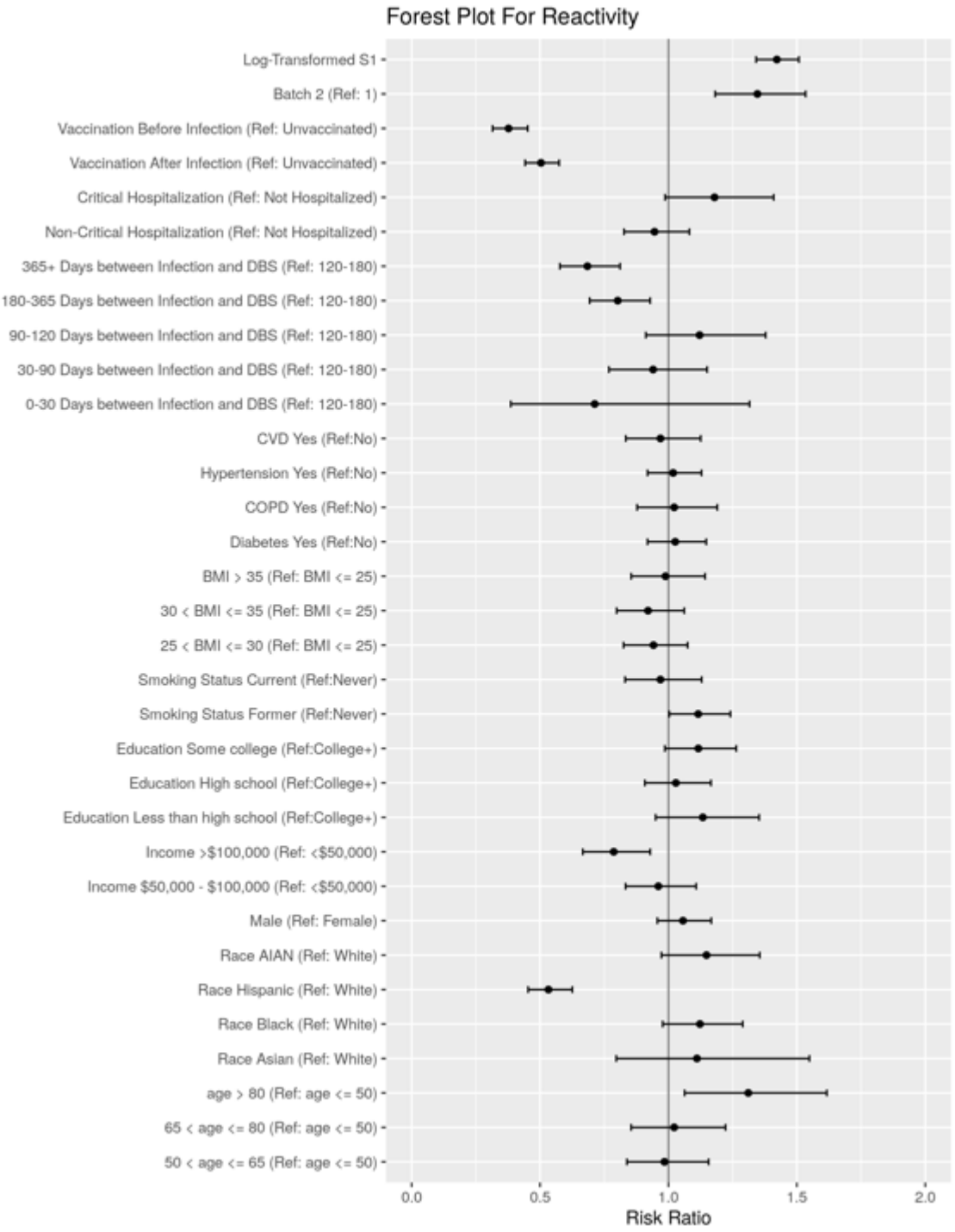
Clinical risk factor	Unadjusted risk ratios (95%Cis) for anti-nucleocapsid reactivity	P-value	Multivariable adjusted risk ratios (95%CIs) for anti-nucleocapsid reactivity	P-value
Age				
Less than 50 years	1.0 (ref)		1.0 (ref)	
50-64 years	1.05 (0.90 to 1.24)	0.54	0.98 (0.84 to 1.16)	0.85
65-79 years	1.19 (1.01 to 1.40)	0.03	1.02 (0.85 to 1.22)	0.81
80 years and greater	1.81 (1.51 to 2.16)	<0.0001	1.31 (1.06 to 1.62)	0.01
Sex				
Female	1.0 (ref)		1.0 (ref)	
Male	1.04 (0.94 to 1.15)	0.47	1.06 (0.96 to 1.17)	0.28
Income				
<50k	1.0 (ref)		1.0 (ref)	
50-100k	1.12 (0.98 to 1.27)	0.10	0.96 (0.83 to 1.11)	0.58
>100k	0.95 (0.81 to 1.11)	0.49	0.79 (0.67 to 0.93)	<0.001
Race/ethnicity				
Non-Hispanic white	1.0 (ref)		1.0 (ref)	
American Indian or Alaskan Native	1.20 (1.06 to 1.37)	<0.01	1.15 (0.97 to 1.36)	0.10
Asian	1.27 (0.90 to 1.79)	0.17	1.11 (0.80 to 1.55)	0.53
Black	1.06 (0.93 to 1.21)	0.37	1.12 (0.98 to 1.29)	0.10
Hispanic	0.61 (0.54 to 0.69)	<0.0001	0.53 (0.45 to 0.63)	<0.0001
Education attainment				
College or beyond	1.0 (ref)		1.0 (ref)	
Less than high school	0.96 (0.82 to 1.13)	0.62	1.13 (0.95 to 1.35)	0.16
High school	1.13 (1.00 to 1.29)	0.06	1.03 (0.91 to 1.17)	0.65
Some college	1.14 (1.00 to 1.30)	0.05	1.12 (0.99 to 1.26)	0.08
Smoking history				
Never	1.0 (ref)		1.0 (ref)	
Former	1.27 (1.15 to 1.41)	<0.0001	1.12 (1.00 to 1.24)	0.04
Current	1.07 (0.92 to 1.26)	0.38	0.97 (0.83 to 1.13)	0.69
Body mass index				
<25 kg/m^2	1.0 (ref)		1.0 (ref)	
25-29.9 kg/m^2	0.85 (0.74 to 0.98)	0.03	0.94 (0.83 to 1.07)	0.37
30-34.9 kg/m^2	0.88 (0.76 to 1.02)	0.09	0.92 (0.80 to 1.06)	0.25
>35 kg/m^2	1.05 (0.91 to 1.21)	0.51	0.99 (0.86 to 1.14)	0.88
Diabetes				
No	1.0 (ref)		1.0 (ref)	
Yes	1.11 (0.99 to 1.23)	0.07	1.03 (0.92 to 1.15)	0.65

Hypertension				
No	1.0 (ref)		1.0 (ref)	
Yes	1.19 (1.08 to 1.31)	<0.001	1.02 (0.92 to 1.13)	0.73
Cardiovascular disease				
No	1.0 (ref)		1.0 (ref)	
Yes	1.07 (0.91 to 1.25)	0.41	0.97 (0.83 to 1.13)	0.68
Chronic obstructive pulmonary disease				
No	1.0 (ref)		1.0 (ref)	
Yes	1.09 (0.93 to 1.26)	0.29	1.02 (0.88 to 1.19)	0.78
Log-transformed anti-S1 MFI (per 1-unit increment)	1.21 (1.15 to 1.28)	<0.0001	1.42 (1.34 to 1.51)	<0.0001
COVID-19 infection severity				
Not hospitalized	1.0 (ref)		1.0 (ref)	
Non-critical hospitalization	1.10 (0.96 to 1.25)	0.18	0.95 (0.83 to 1.08)	0.41
Critical hospitalization	1.41 (1.18 to 1.68)	<0.001	1.18 (0.99 to 1.41)	0.07
COVID-19 vaccine status				
Not vaccinated	1.0 (ref)		1.0 (ref)	
Vaccinated after infection	0.78 (0.70 to 0.88)	<0.0001	0.50 (0.44 to 0.57)	<0.0001
Vaccinated before infection	0.79 (0.67 to 0.93)	0.01	0.38 (0.32 to 0.45)	<0.0001
Time between infection and DBS collection				
120-179 days	1.0 (ref)		1.0 (ref)	
0-29 days	0.45 (0.22 to 0.91)	0.03	0.71 (0.39 to 1.32)	0.32
30-89 days	0.90 (0.73 to 1.10)	0.30	0.94 (0.77 to 1.15)	0.55
90-119 days	1.02 (0.83 to 1.26)	0.85	1.12 (0.91 to 1.38)	0.28
180-364 days	0.82 (0.70 to 0.94)	0.01	0.80 (0.69 to 0.93)	<0.01
>365 days	0.67 (0.58 to 0.78)	<0.0001	0.68 (0.58 to 0.81)	<0.001

FIGURES

Figure 1: Forest plot showing correlates of anti-nucleocapsid antibody reactivity (a) and mean florescence unit (b) among n = 2,154 C4R participants.

a)



b)

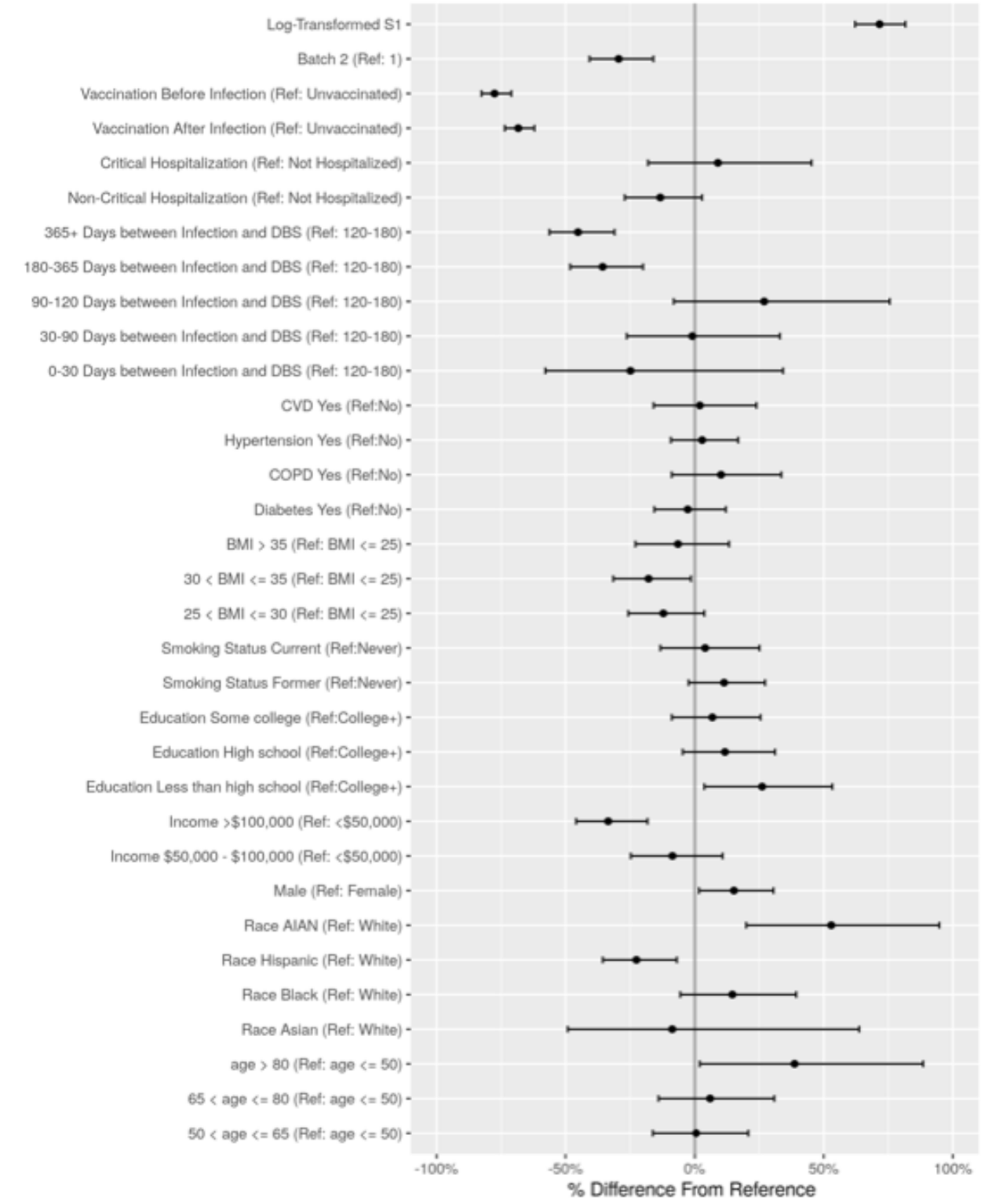
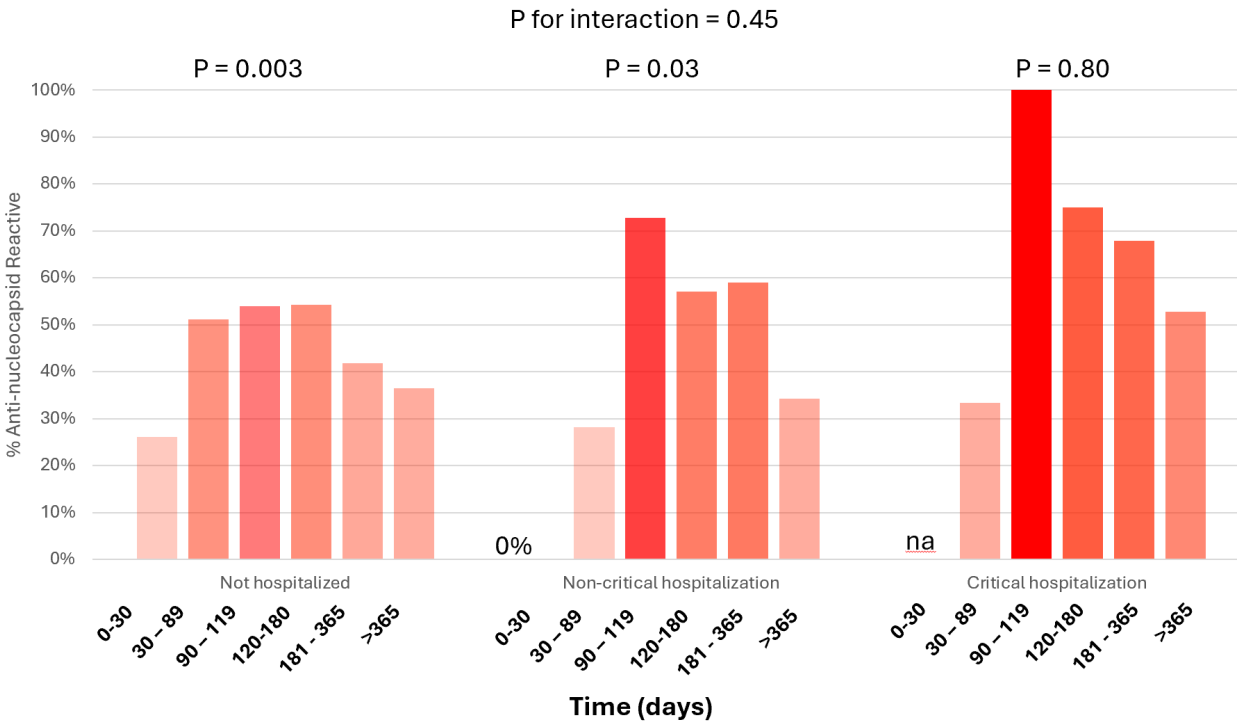
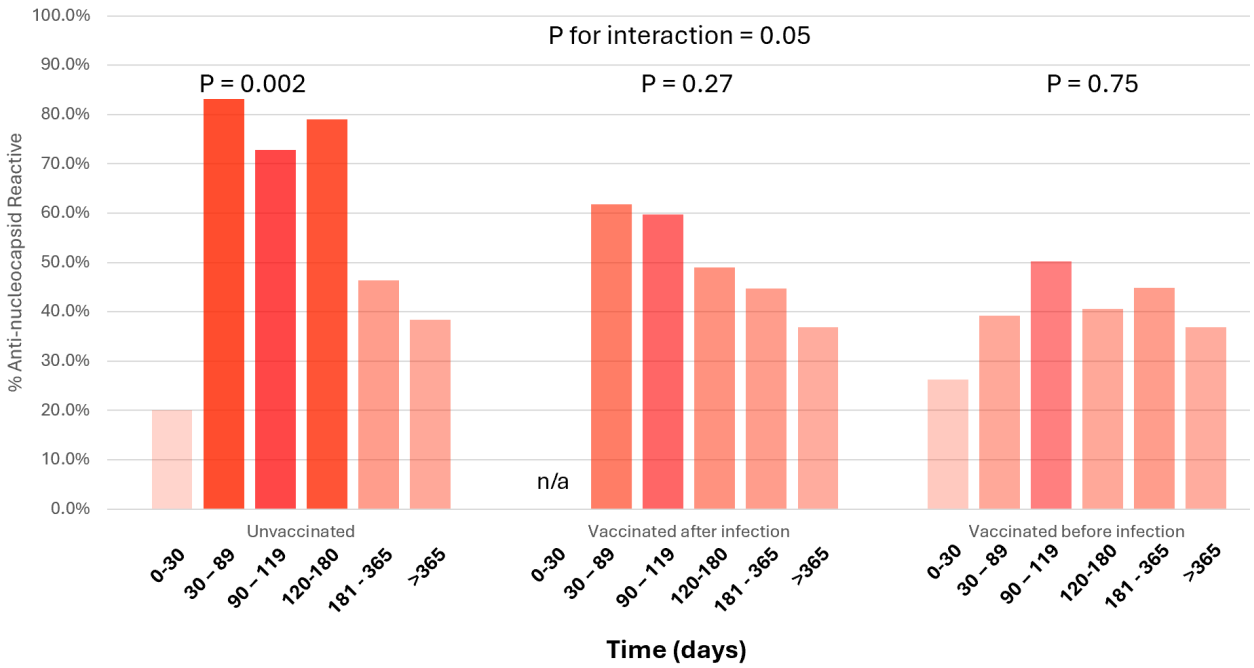

Log-Transformed S1
Batch 2 (Ref: 1)
Vaccination Before Infection (Ref: Unvaccinated)
Vaccination After Infection (Ref: Unvaccinated)
Critical Hospitalization (Ref: Not Hospitalized)
Non-Critical Hospitalization (Ref: Not Hospitalized)
365+ Days between Infection and DBS (Ref: 120-180)
180-365 Days between Infection and DBS (Ref: 120-180)
90-120 Days between Infection and DBS (Ref: 120-180)
30-90 Days between Infection and DBS (Ref: 120-180)
0-30 Days between Infection and DBS (Ref: 120-180)
CVD Yes (Ref:No)
Hypertension Yes (Ref:No)
COPD Yes (Ref:No)
Diabetes Yes (Ref:No)
BMI > 35 (Ref: BMI <= 25)
30 < BMI <= 35 (Ref: BMI <= 25)
25 < BMI <= 30 (Ref: BMI <= 25)
Smoking Status Current (Ref:Never)
Smoking Status Former (Ref:Never)
Education Some college (Ref:College+)
Education High school (Ref:College+)
Education Less than high school (Ref:College+)
Income >$100,000 (Ref: <$50,000)
Income $50,000 - $100,000 (Ref: <$50,000)
Male (Ref: Female)
Race AIAN (Ref: White)
Race Hispanic (Ref: White)
Race Black (Ref: White)
Race Asian (Ref: White)
age > 80 (Ref: age <= 50)
65 < age <= 80 (Ref: age <= 50)
50 < age <= 65 (Ref: age <= 50)
-100%
-50%
0%
50%
100%
% Difference From Reference

Figure 2: Proportion of individuals with anti-nucleocapsid antibody reactivity detected by time since infection according to severity of infection (a) and vaccination status (b) among n=2,154 C4R participants.

a)



b)


P for interaction = 0.05
P = 0.002
P = 0.27
P = 0.75
% Anti-nucleocapsid Reactive
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
n/a
Unvaccinated
Vaccinated after infection
Vaccinated before infection
0-30
30 – 89
90 – 119
120-180
181 - 365
>365
Time (days)