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MAJOR ARTICLE

A Longitudinal Study of Severe Acute Respiratory Syndrome Coronavirus 2 Antibody Response in a Subset of United States Blood Donors

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Background. Blood donors were tested for antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); resulting antibody levels were monitored over time.

Methods. Donors reactive to anti-SARS-CoV-2 spike protein (S1-total antibodies) participated in a follow-up study of 18 months. Testing for nucleocapsid antibodies distinguished between vaccination and infection. Vaccination and symptom information were collected for anti-S1-reactive donors by completing a survey.

Results. The majority of 249 followed donors were over 60 years old (54%), White (90%), and female (58%); 83% had not been vaccinated at enrollment, but by study completion, only 29% remained nonvaccinated. Of the 210 (84%) anti-N-reactive donors, 138 (66%) reported vaccination, whereas 37 (95%) of donors vaccinated and anti-N negative at enrollment remained uninfected. Vaccinated (2 doses) and infected donors showed a steady increase in anti-S1 that increased markedly for vaccinated donors after a booster and infected donors after vaccination (slightly higher for those with hybrid immunity), whereas anti-N levels declined. Most surveyed nonvaccinated donors (65%) reported symptoms, whereas 85% of vaccinated donors were asymptomatic. A coronavirus disease 2019 (COVID-19) diagnosis was reported by 48 (31%) nonvaccinated and 3 (8%) vaccinated donors. Of asymptomatic donors, 38% never tested diagnostically for COVID-19, and 35% tested negative, suggesting an absence of knowledge of the infection.

Conclusions. Healthy blood donors were vaccinated at high rates and remained mostly asymptomatic and noninfected, whereas approximately two thirds of infected donors reported symptoms. Anti-S1 levels increased while anti-N decreased over 18 months but remained comparable between vaccinated and hybrid immune individuals with dramatic anti-S1 increases after vaccination or boosting.

Keywords. COVID-19; infection; SARS-CoV-2; vaccination.

In the early days of the coronavirus disease 2019 (COVID-19) pandemic, when effective treatment against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was lacking, convalescent plasma was used to treat patients hospitalized with COVID-19 [1]. To support the high demand for convalescent plasma and increase the number of presenting blood donors, the American Red Cross (ARC) and many other US blood establishments implemented testing of all donations (referred to as universal testing) for antibodies to SARS-CoV-2 for approximately 12 months [2]. Donations were tested using an assay targeting antibodies to the viral spike (S1) protein, followed by testing anti-S1-reactive donation samples using an assay detecting the nucleocapsid (N) antibody; both assays detect total immunoglobulins (Igs). Although not fully representative of the US population, testing healthy blood donors contributed valuable data on the prevalence of COVID-19 infection, including individuals with asymptomatic infection or milder illness who did not undergo diagnostic testing and were unaware of having been exposed to SARS-CoV-2. Universal testing covered the time from before to after the availability of an authorized vaccine (testing initiated in June 2020 through June 2021). As a result, detecting antibodies against S1 and N during universal testing and the collection of vaccination information provided infection and vaccine trends reflecting population immunity across different demographics and enabled national cross-sectional seroprevalence studies [2–5]. In contrast to cross-sectional evaluations, longitudinal follow up of cohorts of infected and vaccinated donors, including those with hybrid immunity, provides detailed information on the rate of vaccination, development of symptomatic infection, evolution and

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durability of antibody responses, as well as the frequency of re-infection and vaccine breakthrough infections.

In this study, we report seroreactivity dynamics in a subset of blood donors initially identified as anti-SARS-CoV-2 S1 reactive during universal testing and subsequently enrolled in a follow-up study. Additional information about demographics, symptoms, vaccines, and donation motivation was collected via a survey.

METHODS

Study Population and Testing

Between June 15, 2020 and June 25, 2021, the ARC tested every donation from allogeneic donors (ie, excluding those from directed, therapeutic and autologous donors) for antibodies to SARS-CoV-2 using the Ortho VITROS anti-SARS-CoV-2 spike protein S1 total antibody (including IgG, IgA, and IgM) test (Ortho Clinical Diagnostics, Raritan, NJ). The assay, targeting subunit 1 of the spike protein, was used under Emergency Use Authorization (EUA) by the US Food and Drug Administration (100% sensitivity [95% confidence interval, 92.7%–100%] in 49 SARS-CoV-2 [PCR]-confirmed patients 8 days or more after symptoms onset, and 100% clinical specificity [95% confidence interval, 99.1%–100%] in 400 presumed negative individuals) [6]. Blood donors testing anti-S1-reactive by the Ortho assay were eligible to participate in the donor follow-up study, including collection of samples and demographic and survey information. Anti-S1-reactive donation samples were also tested with the Roche Elecsys anti-SARS-CoV-2 test (Roche Molecular Diagnostics, Indianapolis, IN), targeting the N protein, also under EUA (99.5% sensitivity [95% confidence interval, 97%–100%] in 185 samples from symptomatic patients with PCR-confirmed SARS-CoV-2 infection tested ≥14 days post-PCR confirmation, and 99.8% specificity [95% confidence interval, 99.69%–99.88%] in 10 453 samples obtained before December 2019) [7]. These assays have been independently qualified and used extensively [2–5, 8]. In contrast to donations, in which anti-N testing only occurred for anti-S1-reactive samples, each follow-up sample was tested by both anti-S1 and anti-N assays simultaneously. Enrolled donors were asked to provide follow-up samples monthly, and donors with a minimum of 2 COVID-19 tested samples were included in the analysis. Follow-up samples were collected between July 2020 and January 2022, and donors were followed for up to 18 months after their initial anti-S1-reactive result. The data analysis also included testing results from routine blood donations provided by enrolled donors during the study period. Routine vaccination information was collected from donors at the time of donation in combination with the responses to the follow-up survey (see below).

Survey

A survey inquiring about COVID-19 symptoms, information about COVID-19 vaccines, including vaccination date, and pre-existing health conditions that could be associated with the severity of COVID-19 infection was provided to all donors with an anti-S1-reactive result approximately 4 weeks postdonation [2]. The survey also collected information about the donors' knowledge of the ARC COVID-19 testing policy, how they became aware of it, and whether the test contributed to their decision to donate blood. The survey was voluntary and approved by the ARC Institutional Review Board (IRB) without a requirement for written or verbal consent. Donors without an e-mail address or with an incorrect one, requesting no e-mail contact, and Spanish-speaking and less than 18 years old were excluded from the survey. However, none of these criteria affected the eligibility of donors enrolled in the follow-up study. In a previous study of surveyed donors for SARS-CoV-2, less than 2% of donors requested a Spanish-translated survey [2]; thus, only English-language surveys were used for this study.

Donor Consent Statement

For follow-up study enrollment, donors were contacted by e-mail or by phone. For donors who consented or declined by e-mail, this served as the documentation for study participation or refusal. For donors enrolled by phone, authorized staff recorded the consent or refusal on the consent form. All donors received the donor materials and a copy of the consent by mail to take to their follow-up sample appointment. The study protocol and materials were voluntary and approved by the ARC IRB.

Analyses and Statistics

Spaghetti plots were constructed for individual-level Ortho and Roche results using $\log_{10}$-transformed signal-to-cutoff (S/CO) values to document trends over time. Ordinary least square (OLS) regression was used to assess the association between time and the $\log_{10}$-transformed anti-S1 (Ortho) and anti-N (Roche) S/CO levels. The fitted intercept, slope, and the measure of goodness of fit (R^2) resulting from the OLS were also determined. All analyses were performed in SAS software version 9.4 (SAS Institute Inc., Cary, NC). *P* values of less than .05 were considered significant, and relevant evaluations were 2-sided. Demographics were compared between symptomatic and asymptomatic respondents using the G-test of independence. Post hoc Fisher's exact tests were performed on categories with significant G-test results to isolate within-group differences comparing each group proportion to all other groups with significance assessed using the Bonferroni correction for multiple comparisons. Logistic regression was used to compare respondents' vaccination status with a report of any listed symptoms and motivation for donating.

RESULTS

A total of 249 donors testing reactive for anti-SARS-CoV-2 S1 were enrolled in the follow-up study (Figure 1*A*). By the end of

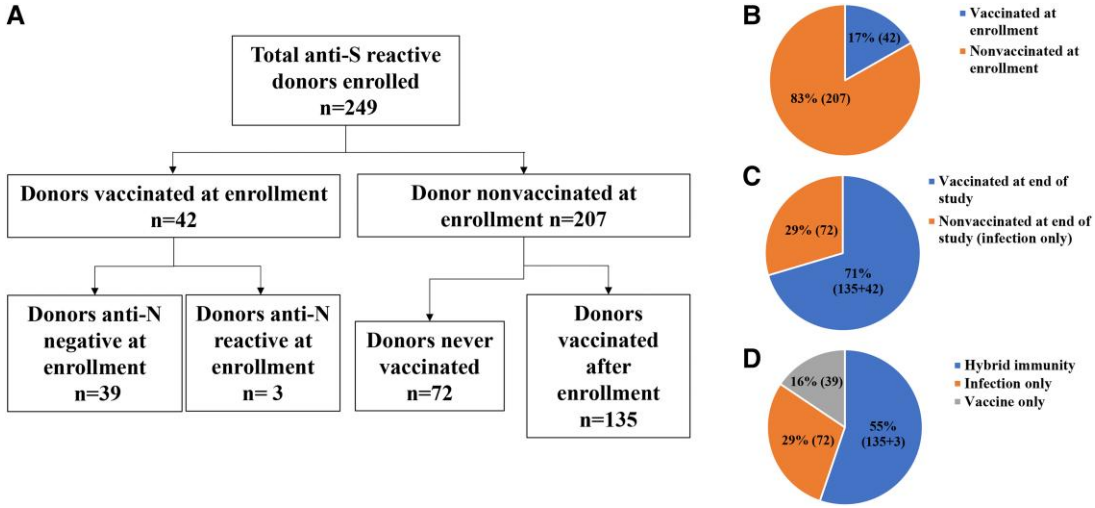

Figure 1. Summary of donors enrolled in the follow-up study. A total of 249 donors with a reactive anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) S1 total immunoglobulin (Ig) assay (Ortho) were enrolled (*A*). Donations were also tested for anti-SARS-CoV-2 nucleocapsid protein (N) using a total Ig assay (Roche). At enrollment, 17% of the 249 donors ($n = 42$) had received a vaccine (*B*), whereas an additional 135 donors received SARS-CoV-2 vaccination after enrollment, with 71% ($n = 135 + 42 = 177$) having been vaccinated by the end of the study (*C*). Of the total 249 donors enrolled in the study, 16% ($n = 39$) were vaccinated and never infected, 55% ($n = 135 + 3 = 138$) were infected and vaccinated (hybrid immunity), and 29% ($n = 72$) were infected but remained nonvaccinated (*D*). Thus, at study enrollment, 84% ($207 + 3 = 210$) were infected.

the study, we had collected and tested 2492 samples from the enrolled donors. Forty-two (17%) of the 249 donors reported vaccination before their first reactive test, whereas 207 (83%) were nonvaccinated at enrollment (Figure 1*B*). Anti-S1-reactive samples were also tested for anti-SARS-CoV-2 N. Antibodies to N are produced after SARS-CoV-2 infection but not after vaccination, using the vaccine authorized in the United States at the time of the study. This allowed discrimination between vaccinated individuals without infection and those with a hybrid immunity (vaccine and infection). Of the 42 vaccinated donors, 39 (93%) were nonreactive for anti–SARS-CoV-2 N and were considered the vaccine-only group. The remaining 3 vaccinated donors (7%) were included in the hybrid immunity group. Only 2 of the 39 anti-N-nonreactive donors had reactive anti-N results on a follow-up sample, thus 95% remained uninfected. In both cases, the infection came before the booster vaccination, approximately 8 months after the initial dose of the vaccine. The rate of vaccine breakthrough infections was very low. Of the 207 (83%) donors with only natural immunity (nonvaccinated) at enrollment, 135 (65%) received the COVID-19 vaccine during the study, whereas 72 (35%) remained unvaccinated; adding these 207 with natural immunity to the 42 (39 + 3) donors vaccinated at enrollment yields the 249 total (Figure 1*C*). Of the 249 enrolled donors by the end of the study, 39 (16%) were vaccinated only and never infected, 138 (135 + 3, 55%) were infected and vaccinated (hybrid immunity), and 72 (29%) remained nonvaccinated (Figure 1*D*). All 207 nonvaccinated donors were infected (anti-N-reactive) except 1 donor, who tested anti-N nonreactive on their initial anti-S1-reactive donation sample. The initial sample from 5 donors was not tested for anti-N due to a lack of residual volume. All 6 nonvaccinated donors (5 anti-N not-tested and 1 initially anti-N nonreactive) tested anti-N reactive on their first follow-up sample. In total, at least 204 ($207 - 6 = 201 + 3$, 82%) donors were infected at enrollment, whether vaccinated or not, and, subsequently, the number increased to 210 ($207 + 3$, 84%). Most donors vaccinated at enrollment received 2 doses of an mRNA vaccine (12 Pfizer and 17 Moderna). Four donors received a single-dose vaccine (J&J), including 1 who was anti-N reactive at index. Nine donors did not disclose the vaccine type but reported 2 doses, suggesting they received an mRNA vaccine.

Most donors were over 60 years old (54%), White (90%), and female (58%) (Table 1). Overall, the demographics of followed donors were comparable to the blood donor population who tested anti-S1-reactive during universal screening. However, most donors who received the vaccine before their qualifying donations (79%) were older than 60 years, and none were younger than 39, reflecting the age-dependent roll-out of vaccines. Donors who received the vaccine after enrollment followed a similar trend: of 101 initially nonvaccinated donors over 60 years old, 72% ($n = 73$) received the vaccine during the study, compared to 61% ($n = 51$) of the 40 to 59 age group and 50% ($n = 11$) of the 18 to 39 age group.

Qualifying samples for vaccine-only donors ($n = 39$) were collected between February and August 2021. Almost all donors in this group (90%) received their vaccine between January and March 2021. Vaccination dates for donors in the hybrid immunity group, who received the vaccine after infection, were available for 125 of the 135 donors. Similar to the

Table 1. Demographic Characteristics of Blood Donors Enrolled in the Study

Characteristics	S+ Blood donors $n = 1\,075\,243$	Vaccinated $n = 42$	Nonvaccinated $n = 207$	Total $n = 249$
Sex	No. (%)	No. (%)	No. (%)	No. (%)
Female	597 998 (56)	17 (40)	127 (61)	144 (58)
Male	477 245 (44)	25 (60)	80 (39)	106 (42)
Age				
18–39 years	270 290 (26)	0	22 (11)	22 (9)
40–59 years	412 802 (38)	9 (21)	84 (40)	93 (37)
≥60 years	383 151 (36)	33 (79)	101 (49)	135 (54)
Race				
African American	24 964 (2)	2 (5)	10 (5)	12 (5)
Asian	26 544 (2)	1 (2)	1 (0.5)	2 (1)
American Indian	2866 (0.3)	0	1 (0.5)	1 (0.5)
White	961 257 (89)	38 (91)	187 (90)	226 (90)
Hispanic	40 625 (4)	0	8 (4)	8 (3)
More than 1 race	9976 (1)	1 (2)	1 (0.5)	1 (0.5)

vaccine-only donors, most received a vaccine by March 2021 (74%), with 54 of 125 (43%) receiving the vaccine in March alone, probably relating to increased vaccine access.

Of the 249 enrolled donors, 195 (78%) completed a survey designed to collect SARS-CoV-2-related information, such as disease symptoms, diagnosis, and underlying risk factors (Tables 2, 3, and 4). Symptoms were reported by most nonvaccinated donors 14 or more days before donation ($n = 102$; 65%) (Table 2), whereas only 7 donors of the vaccinated group (18%) reported symptoms they believed to be associated with COVID-19 infection (odds ratio [OR] = 8.2; $P < .01$). However, only 2 of the 7 donors were anti-N-reactive at index and reported a COVID-19 diagnosis. The remaining 5 donors were never reactive to anti-N-antibodies, suggesting that the symptoms may have been related to vaccination or a different infection. A third vaccinated donor with an anti-N-reactive test at enrollment and a diagnosis of COVID-19 infection did not report any symptoms. Fifty-five (35%) infected and surveyed donors nonvaccinated at enrollment did not report symptoms. Of those, only 15 (27%) had a positive diagnostic test and were aware of their infection. Of the remaining 40, 21 (38%) never received a COVID-19 diagnostic test, and 19 (35%) had a negative diagnostic test, suggesting they were unaware of being infected before receiving the test results provided by the ARC. The most common symptoms reported were fatigue (42%), myalgia (29%), and headache (28%). Furthermore, nonvaccinated respondents were more likely to be diagnosed with COVID-19 (OR = 5.1; $P < .01$). In fact, COVID-19 diagnosis was reported by 48 (31%) nonvaccinated donors and only 3 vaccinated donors (2 with symptoms and 1 without symptoms).

Demographics were compared between symptomatic and asymptomatic survey responders, with an overall significant difference found between the 2 groups by age ($P < .01$), where higher rates of those reporting symptoms occurred in the 40- to 59-year-old group (46% symptomatic vs 27%; asymptomatic; $P = .02$), but higher rates of those without symptoms were reported for those over 60 years (67% asymptomatic vs 42% symptomatic; $P < .01$) (Table 3). The increasing frequency of vaccination and subsequent protection from the virus in the older age group likely drove the significant difference.

Pre-existing conditions were reported by 71 (36%) of the total donors, with 60 (31%) indicating high blood pressure and 16 (9%) with diabetes (Table 4). The survey also inquired about donor awareness of the COVID-19 universal testing performed by the ARC and whether testing contributed to the decision to donate blood. Unvaccinated respondents were more likely to have donated to receive an antibody test (OR = 9.2; $P < .01$), and although most donors were aware of the test ($n = 154$, 79%), only 55 (28%) reported the test as a motivation to donate blood (Table 4).

The geographic distribution of donors enrolled in the follow-up study by zip code of residence followed a similar pattern to blood collections. States with the highest number of donors tested during SARS-CoV-2 universal screening included California, Georgia, Ohio, North Carolina, New York, Maryland, Michigan, Minnesota, Pennsylvania, and Virginia. Each state included at least 10 donors enrolled in the follow-up study. Maryland was an exception, with 28 donors enrolled in the study, including 21 from the vaccine-only group.

Anti-S1 reactivity in most of the 39 vaccine-only donors (anti-N-nonreactive at enrollment) remained elevated during the observation period (Figure 2*A*). Levels varied from donor to donor and were lower in recipients of the J&J vaccine. In donors who received a booster ($n = 23$) (Figure 2*B*), anti-S antibodies increased substantially, reaching comparable levels. Two of three J&J vaccine recipients who received a booster (1 J&J and 1 mRNA) displayed a spike in anti-S reactivity. The third recipient of the J&J vaccine did not receive a booster.

In addition to the 72 donors who remained nonvaccinated, prevaccination samples were available from 105 of the 135 blood donors who received a vaccine after enrollment. This cluster of samples from 177 donors represents the infection-only group. Testing of prevaccination samples from these donors showed a steady increase in the anti-S1 signal levels ($P < .0001$) during the observation period (up to 18 months), whereas anti-N showed steady declining levels ($P < .0001$) (Figure 3). Some donors showed fluctuating levels of anti-N, possibly due to reinfection. However, the actual number of reinfections could not be determined because increased anti-N reactivity was often of short duration and was not strongly associated with increased anti-S levels, which remained substantial over time.

Postvaccination blood samples were available from 122 of the 135 nonvaccinated donors who received the vaccine during the study period. After vaccination, the 122 previously infected

Table 2. Symptoms and COVID-19 Diagnosis Reported by Blood Donors Enrolled in the Study Through a Survey

	Vaccinated ($n=38$)	Nonvaccinated ($n=157$)	Total ($n=195$)	Significance
Symptoms	No. (%)	No. (%)	No. (%)	…
No	31 (82)	55 (35)	86 (44)	$P<.01$
Yes	7 (18)	102 (65)	109 (56)	OR = 8.2
Fever	0	32 (20)	32 (16)	…
Headache	1 (3)	53 (34)	54 (28)	…
Sore throat	1 (3)	33 (21)	34 (17)	…
Chest congestion	0	36 (23)	26 (13)	…
Cough	4 (11)	45 (29)	49 (25)	…
Fatigue	4 (11)	77 (49)	81 (42)	…
Myalgia	3 (8)	53 (34)	56 (29)	…
Short of breath	0	25 (16)	25 (13)	…
Nausea vomiting diarrhea	0	27 (17)	27 (14)	…
Runny nose	4 (11)	38 (24)	42 (22)	…
Loss of taste	0	37 (24)	37 (19)	…
Loss of smell	1 (3)	44 (28)	45 (23)	…
COVID-19 dx. No. (%)	…	…	…	…
No	35 (92)	109 (69)	144 (74)	$P<.01$
Yes	3 (8)	48 (31)	51 (26)	OR = 5.1

Only significant comparisons were indicated.

Table 3. Demographics of Symptomatic and Asymptomatic Survey Responders

	Symptoms ($n=109$)	No Symptoms ($n=86$)	Total ($n=195$)	Significance
Sex	No. (%)	No. (%)	No. (%)	…
Female	65 (60)	43 (50)	108 (55)	…
Male	44 (40)	43 (50)	87 (45)	…
Age	…	…	…	$P<.01$
18–39 years	13 (12)	5 (6)	18 (9)	$P=.63$
40–59 years	50 (46)	23 (27)	73 (37)	$P=.02$
≥60 years	46 (42)	58 (67)	104 (53)	$P<.01$
Race				
African American	6 (5)	2 (2)	8 (4)	…
Asian	0	1 (1)	1 (1)	…
American Indian	0	0	0	…
White	97 (89)	81 (94)	178 (91)	…
Hispanic	5 (5)	2 (2)	7 (4)	…
More than 1 race	1 (2)	0	1 (0.5)	…

Only significant comparisons were indicated.

Table 4. Pre-existing Conditions Reported by Blood Donors Enrolled in the Study Through a Survey

	Vaccinated ($n=38$)	Nonvaccinated ($n=157$)	Total ($n=195$)	Significance
Health Conditions, No. (%)				
No	25 (66)	99 (63)	124 (64)	…
Yes	13 (34)	58 (37)	71 (36)	…
Asthma	1 (3)	8 (5)	9 (5)	…
Cardiovascular disease	0	3 (2)	3 (2)	…
Diabetes	2 (5)	14 (9)	16 (9)	…
High blood pressure	12 (32)	48 (31)	60 (31)	…
Weakened immune system	0	4 (3)	4 (2)	…
COVID-19 testing aware				
No/no answer	11 (29)	30 (19)	41 (21)	…
Yes	27 (71)	127 (81)	154 (79)	…
ARC e-mail	15 (56)	58 (46)	33 (21)	…

Table 4. Continued

	Vaccinated ($n = 38$)	Nonvaccinated ($n = 157$)	Total ($n = 195$)	Significance
ARC app	13 (48)	39 (31)	52 (34)	…
ARC SMS	4 (15)	4 (3)	8 (5)	…
ARC phone call	0	7 (6)	7 (5)	…
Media	2 (7)	12 (9)	14 (9)	…
Internet	1 (4)	21 (17)	22 (14)	…
Social media	0	4 (3)	4 (3)	…
Friends and family	1 (4)	24 (19)	25 (16)	…
Motivation				
No/no answer	36 (95)	104 (66)	140 (72)	$P < .01$
Yes	2 (5)	53 (34)	55 (28)	OR = 9.2

Abbreviations: ARC, American Red Cross; COVID-19, coronavirus disease 2019; dx, diagnosis; OR, odds ratio; SMS, Short Message Service.
Only significant comparisons were indicated.

donors showed an increase in anti-S signal levels (Figure 4*A*), comparable with the levels reached by vaccinated donors after receiving the booster ($n = 23$) (Figure 2*B*). Compared with the vaccine-only group ($n = 39$), previously infected donors who received the vaccine ($n = 135$) developed slightly higher anti-S1 signal levels after vaccination (Figure 4*B*).

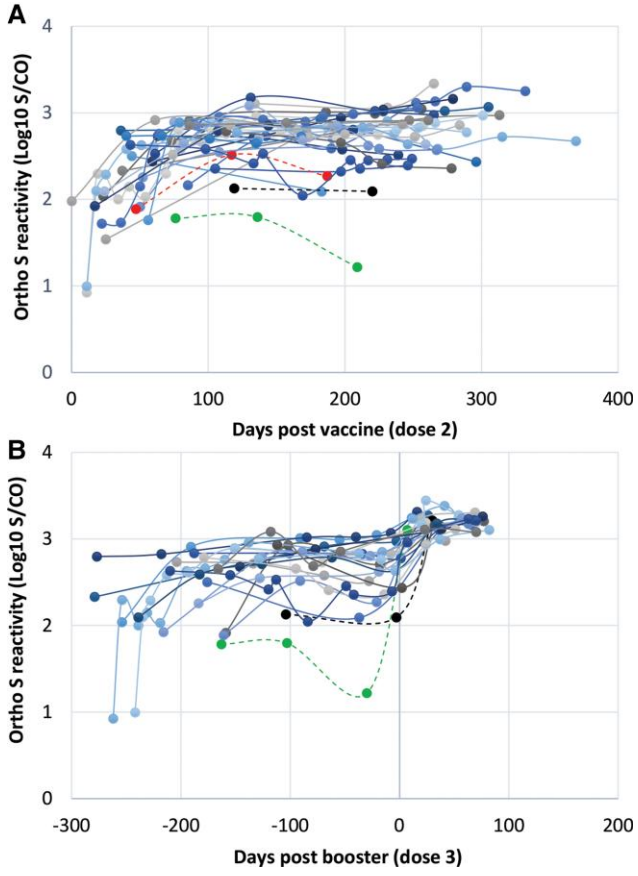

Figure 2. Vaccine-only antibody response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) anti-S1 before and after the booster dose. Of the 42 donors vaccinated at enrollment, the 3 anti-N-reactive donors were excluded from this analysis to show responses of only noninfected donors. Most donors received an mRNA vaccine (Pfizer or Moderna); 3 donors who received the J&J vaccine are represented with a dashed line. (*A*) The SARS-CoV-2 S response to vaccination before receiving a booster. (*B*) Of the 39 vaccine-only donors (anti-N-nonreactive), 23 received a booster. S/CO, signal-cutoff.

DISCUSSION

The primary purpose of this study was to investigate the variation in markers of infection and vaccination over time among a population of blood donors. We detected a steady upward trend of total Ig anti-S1 antibodies for up to 18 months, whereas anti-N antibodies showed a steady decline, as previously described for both assays in our blood donor population for 4–6 months [2] and the latter as previously described for over 20 months using the same assay [9]. Although the decrease in protection against reinfection over time is well documented in vaccinated and infected individuals, the stability and functionality of anti-S1 were reported in blood donors up to 1 year after infection [2, 10–13]. It is important to mention that antibody detection does not provide information about neutralizing activity or protection from reinfection. Furthermore, both assays used in this study are pan-Ig tests, detecting total Ig, which have been shown to detect higher antibody levels than IgG-, IgA-, and IgM-specific tests [14]. Anti-S1 seroreactivity induced by RNA vaccines and infection were comparable, albeit with some variability from donor to donor. An additional dose of the vaccine (booster) induced a marked increase in the levels of anti-S1, virtually eliminating the variability between individuals and bringing the seroreactivity to higher levels. After receiving a vaccine, previously infected donors (hybrid immunity) displayed the same marked and homogeneous surge in anti-S1 levels with a steady increase over time. In addition, when the levels of anti-S1 after vaccination in vaccine-only (before booster) and hybrid immunity donors were compared, the hybrid immunity antibody levels were initially modestly higher. The difference disappeared 9 months after the initial vaccine dose when most vaccine-only donors received a booster inoculation. Indeed, the average number of days between the initial dose of vaccine and the booster inoculation was 250 days (range, 207–316 days). These data suggest that the immune response induced

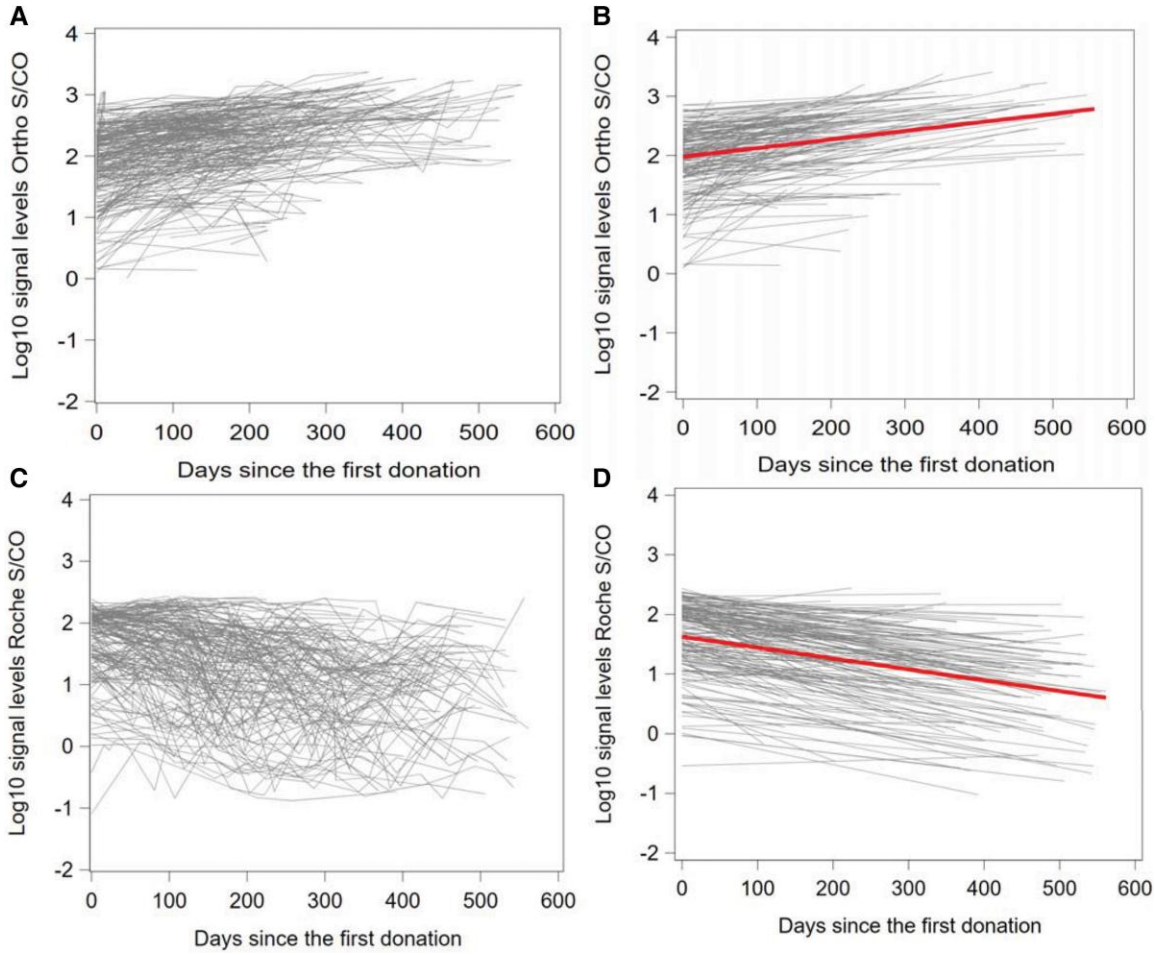

Figure 3. Severe acute respiratory syndrome coronavirus 2 anti-S1 (*A* and *B*) and anti-N (*C* and *D*) antibody response over time in those with viral infection only. Before vaccination, donation testing data were available for 177 donors ($n = 72$ never vaccinated +105 from whom prevaccination samples were available in the 135 subsequently vaccinated). (*A*) shows donor anti-S1 levels have an increasing trend over time, whereas (*C*) shows donor anti-N levels have a decreasing trend over time. The ordinary least square regressions in (*B*) and (*D*) indicate changes in anti-S1 and anti-N $\log_{10}$-transformed signal-cutoff (S/CO) levels over time (in days) for each donor. The bold lines are the best-fitted lines derived from the combined data set, whereas each gray line is the fitted regression line for each donor over time. (*B*) shows a significant increase in anti-S1 levels ($P < .0001$; $R^2 = 0.1043$; slope = 0.00145). Pearson correlation further indicates a positive and weak correlation between the anti-S1 level and time ($r = 0.3230$, $P < .0001$). In contrast, (*D*) shows a significant decrease in anti-N levels ($P < .0001$; $R^2 = 0.1140$; slope = −0.00183). Pearson correlation further indicates a negative and weak correlation between the anti-N level and time ($r = -0.3377$, $P < .0001$).

by hybrid immunity compared to that induced by the 2 doses of the vaccine plus a booster dose were equivalent. Similar results were obtained using an anti-S IgG assay with higher levels of antibodies after vaccination identified among those with hybrid immunity versus vaccination only, and this difference remained for 11 months or until subsequent booster [15]. Although it is unknown whether sustained anti-S1 levels translate to protection, increased effectiveness of hybrid immunity was previously reported against BA.1 and BA.2 [16] and against Omicron and Delta [17]. Fluctuation in the levels of anti-N was noted in a small number of donors. Although reinfection is possible, the determination was complicated by the absence of a clear definition of anti-N dynamics (waning and boosting) in assessing reinfection in anti-N-reactive donors and the absence of a reliable correlation with concomitant increases in anti-S1 [9]. Therefore, a more likely explanation could be the variability of the anti-N assay used in this study. In addition, follow-up donations were collected before the appearance of the Omicron variant, responsible for many reinfections in the United States due to limited protection provided by the original vaccines [18]. Two breakthrough infections were identified in vaccinated donors who developed anti-N during the study. In both cases, reinfection occurred more than 200 days after their second dose of vaccine and in the absence of a booster dose.

Data collected from the survey represent a unique strength of this study, because they allowed for an in-depth connection between antibody test results, donor vaccination status, and symptoms. A large proportion of surveyed, previously infected donors (65%) reported COVID-19-related symptoms, whereas 82% of those who were vaccinated did not report symptoms.

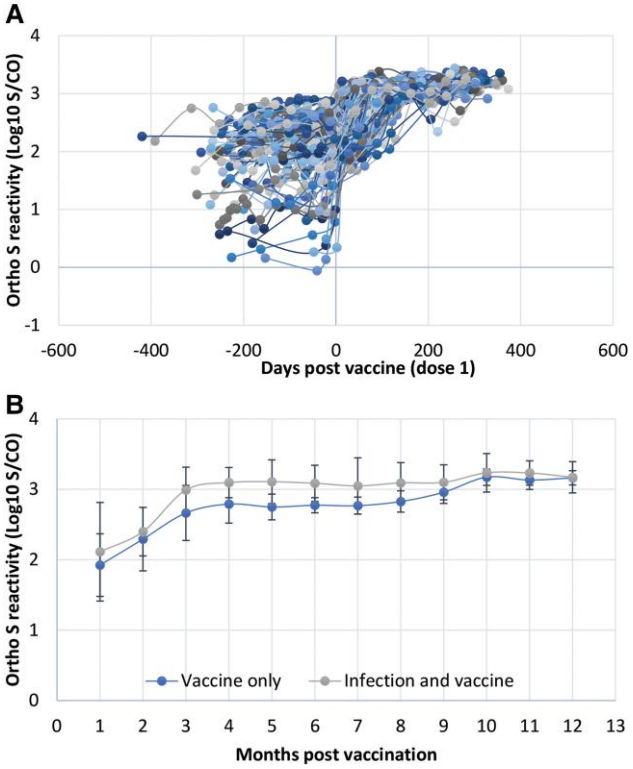

Figure 4. Antibody response before and after vaccination in donors infected before study enrollment. (*A*) Antisevere acute respiratory syndrome coronavirus 2 anti-S1 response to vaccination after viral infection. Of the 135 donors who received the vaccine after enrollment, 122 had postvaccine tested donations. (*B*) Anti-S1 signal levels after vaccination in vaccine-only donors ($n = 39$) and donors with hybrid immunity ($n = 135$). S/CO, signal-cutoff.

Together with the low number of anti-N positive vaccinated donors (3 of 42), these data suggest that the vaccine provided substantial protection against infection and subsequent disease. The most common symptoms were fatigue, headache, and myalgia, in agreement with previously reported data [2]. Nevertheless, 55 (35%) of previously infected donors who completed the survey did not report experiencing COVID-19-related symptoms. A portion (27%) had a positive result by swab or saliva test, but the majority either were not tested or tested negative for the virus. Regardless of whether the donors were aware of being exposed to the virus, these donors experienced an asymptomatic infection. In several meta-analysis studies, researchers have attempted to estimate the rate of asymptomatic SARS-CoV-2 infections and their contribution to the transmission of the virus in the community. However, the numbers vary greatly depending on geographic location, study population, and time of sample collection [19, 20]. At the same time, the transmission of the virus by people with asymptomatic infection has been well documented [21]. Although blood donors do not accurately represent the general population, studies such as this may help provide a more reliable representation of the proportion of asymptomatic infections. Indeed, SARS-CoV-2 antibodies were detected by universal testing, independent of discriminating factors such as donor demographics, geographic residence, and history of COVID-19 infection. Furthermore, because the time between the qualifying reactive donation and the completion of the survey was a minimum of 3 weeks, it is unlikely for these cases to be presymptomatic infections and more likely to be truly asymptomatic.

Compared with the blood donor population that tested anti-S1 reactive during universal screening, the follow-up study donors showed a similar distribution in sex, age, and race. One exception was the age of vaccine-only donors (anti-S1 reactive/anti-N negative), who were mostly over 60 years old (79%), and none was younger than 50 years. The vaccine roll-out phases are a likely factor in this trend. Indeed, although the initial reactive donation for vaccine-only donors was collected between February and August 2021, almost all donors in this group (90%) received their vaccine between February and March of the same year. At that time, vaccine availability across the United States was mainly limited to individuals in phase 1, which included persons ≥65 years of age. Similarly, nonvaccinated donors who were initially identified as anti-S1 reactive due to a previous infection and received the vaccine during the study were overwhelmingly 60 years or older (72%). By the end of the study, 71% of the enrolled donors had received a vaccine, a proportion much higher than the general population.

One limitation of this study is the small sample size; however, using a limited number of donors allows for a detailed analysis of those individuals, and the sample size was robust ($n = 2492$ samples). In addition, we did not have a specific timeframe for when the infection occurred, although we knew when vaccination occurred; we did not define PCR confirmation as the point of infection.

CONCLUSIONS

In conclusion, we detected mildly increasing levels of anti-S antibodies for up to 18 months, regardless of whether anti-S1 was acquired by multiple vaccinations or infection, whereas anti-N antibody levels slightly declined (albeit both changes were significant). Vaccinations provided adequate protection, whereas vaccination boosters and vaccination received after natural infection induced a rapid and marked increase in anti-S1 levels. Our study further showed that asymptomatic infections in previously infected healthy blood donors were common, emphasizing the importance of vaccination in protecting from SARS-CoV-2 infection or disease. The analysis of over 2400 data points from 249 blood donors provides valuable insight into the immune response dynamics after a SARS-CoV-2 infection and vaccination.

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