RESEARCH LETTER

Prevalence and Durability of SARS-CoV-2 Antibodies Among Unvaccinated US Adults by History of COVID-19

As of December 28, 2021, approximately 27% of the US population was unvaccinated against SARS-CoV-2,[1] yet the prevalence of natural immunity remains unknown. Blood donor studies may have selection bias and lack clinical information.[2] Previous COVID-19 infection is a possible surrogate for natural immunity, but 1 study suggested that 36% of COVID-recovered individuals are serologic nonresponders.[3] Even among individuals who develop antibodies, durability of this response beyond 6 months remains unknown. We characterized natural immunity and long-term durability among unvaccinated individuals using anti-spike antibodies, the first line of defense against SARS-CoV-2.

Methods | Healthy adults who reported no SARS-CoV-2 vaccination were recruited via 1 public Twitter post and 1 public Facebook advertisement between September 11, 2021, and October 8, 2021. Participants completed an online questionnaire about demographics, COVID-19 status, and mask use. Using weighted random sampling (relative weights based on the estimated unvaccinated US population by age, race and ethnicity, and education[1]), we created 3 equally sized sample groups among those who reported a test-confirmed COVID-19 infection ("COVID-confirmed"), who believed they had COVID-19 but were never tested ("COVID-unconfirmed"), and who did not believe they ever had COVID-19 and never tested positive ("no-COVID"). These groups were invited to undergo antibody testing at LabCorp facilities nationwide.

Qualitative detection of antibodies against the SARS-CoV-2 antinucleocapsid (N) protein (positive cutoff index ≥1.0) and semiquantitative detection of antibodies against the

Table. Population Characteristics and Antibody Result Stratified by COVID-19 Diagnosis, Confirmed or Suspected

		No. (%)			
		COVID-19 infection			
	Total, No. (%)	Confirmed	Unconfirmed	Believes never had COVID-19	*P* value[a]
No.	816	295	275	246	
Age, median (IQR), y	48 (37-59)	47 (37-59)	48 (37-58)	49 (38-62)	.49
Men	395 (48)	140 (47)	132 (48)	123 (50)	.83
Women	421 (52)	155 (53)	143 (52)	123 (50)	
Race[b]					
African American/Black	12 (2)	4 (1)	7 (3)	1 (0.4)	.01
Asian	35 (4)	16 (5)	12 (4)	7 (3)	
White	669 (82)	228 (77)	221 (80)	220 (89)	
Other	100 (12)	47 (16)	35 (13)	18 (7)	
Hispanic ethnicity[b]	106 (13)	43 (15)	39 (14)	24 (10)	.40
Attended college	518 (64)	179 (61)	162 (59)	177 (72)	.004
Mask use					
Routinely	114 (14)	53 (18)	28 (10)	33 (13)	<.001
Sometimes	214 (30)	103 (35)	76 (28)	68 (28)	
Rarely	355 (44)	117 (40)	122 (44)	116 (47)	
Never	100 (12)	22 (8)	49 (18)	29 (12)	
Nucleocapsid-positive[c]	440 (54)	280 (95)	138 (50)	22 (9)	<.001
Anti-RBD-positive	471 (58)	293 (99)	152 (55)	26 (11)	<.001
Antinucleocapsid/anti-RBD agreement	779 (95)	248 (96)	219 (92)	215 (98)	<.001
Anti-RBD, U/mL[c]					
Median (IQR)	158 (52-499)	205 (61-535)	131 (35-402)	82 (19-172)	.005
≥250	185 (23)	129 (44)	50 (18)	6 (2)	<.001
≥500	117 (14)	79 (27)	33 (12)	5 (2)	<.001
≥1000	63 (8)	43 (15)	16 (6)	4 (2)	<.001
Days since COVID-19 diagnosis, median (IQR)[c]		261 (56-387)			

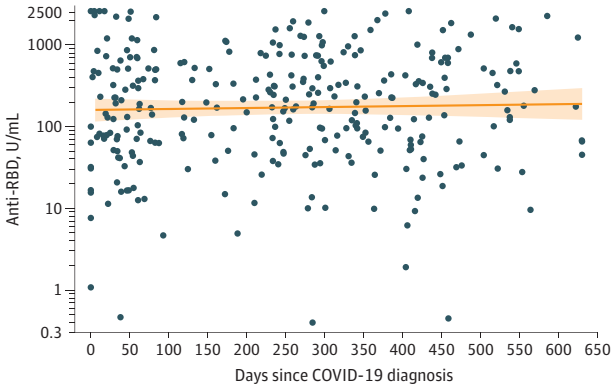
Abbreviation: RBD, receptor-binding domain.

[a] A χ^2 test was used for categorical variables (Fisher exact test for rare outcomes) and a Wilcoxon rank sum test for continuous variables.

[b] Race and ethnicity data were collected to perform weighted random sampling among the 3 groups for antibody testing. Participants could select from predefined categories of African American/Black, Asian, White, or other. Ethnicity was self-reported. Participants could select among predefined categories of Hispanic/Latino yes/no.

[c] Among participants with positive titers.

Figure. Anti-Spike RBD Levels by Time Since COVID-19 Diagnosis



Anti–receptor-binding domain (RBD) levels did not differ by months since COVID-19 diagnosis (0.8% increase [95% CI, −2.4% to 4.2%] per month; $P = .62$). Data markers indicate individual anti-RBD titers; solid orange curve with shaded area, linear regression with 95% confidence range.

SARS-CoV-2 spike protein receptor-binding domain (RBD) (positive cutoff ≥0.8 U/mL) were performed (Elecsys; Roche Diagnostics International Ltd). Various cutoffs are reported (≥250 U/mL, ≥500 U/mL, ≥1000 U/mL) based on reported associations with neutralization.[4]

Population characteristics were compared using χ^2 test for categorical (Fisher exact test for rare outcomes) and Wilcoxon rank sum test for continuous variables. We used linear regression to analyze the association between time after infection and log antibody titer. The threshold for statistical significance was $P < .05$ (2-sided). All analyses were performed using Stata 17.0/SE (StataCorp). The study was approved by the Johns Hopkins institutional review board. Participants provided electronic informed consent.

Results | Of 1580 individuals invited to undergo serologic testing, 816 (52%) did so between September 24, 2021, and November 5, 2021. Participants had a mean age of 48.0 years, 421 (52%) were women, and 669 (82%) were White (**Table**). Fourteen percent reported routine mask use in public. Anti-RBD and anti-N antibody presence/absence were correlated (95%; Cohen κ=0.908).

Among 295 reported COVID-confirmed participants, 293 (99%) tested positive for anti-RBD antibodies (≥250 U/mL, 44%; ≥500 U/mL, 27%; ≥1000 U/mL, 15%). A median of 8.7 (IQR, 1.9-12.9; range, 0-20) months passed since reported COVID-19 diagnosis. The median anti-RBD level among those who tested positive was 205 (IQR, 61-535) U/mL. There was no evidence of association between time after infection and antibody titer (0.8% increase [95% CI, -2.4% to 4.2%] per month; $P = .62$) (**Figure**).

Among 275 reported COVID-unconfirmed participants, 152 (55%) tested positive for anti-RBD antibodies (≥250 U/mL, 18%; ≥500 U/mL, 12%; ≥1000 U/mL, 6%). The median level among those who tested positive was 131 (IQR, 35-402) U/mL.

Among 246 reported no-COVID participants, 11% tested positive for anti-RBD antibodies (≥250 U/mL, 2%; ≥500 U/mL, 2%; ≥1000 U/mL, 2%). The median level among those who tested positive was 82 (IQR, 19-172) U/mL.

Discussion | In this cross-sectional study of unvaccinated US adults, antibodies were detected in 99% of individuals who reported a positive COVID-19 test result, in 55% who believed they had COVID-19 but were never tested, and in 11% who believed they had never had COVID-19 infection. Anti-RBD levels were observed after a positive COVID-19 test result for up to 20 months, extending previous 6-month durability data.[5]

Study limitations include lack of direct neutralization assays, the fact that antibody levels alone do not directly equate to immunity,[4,6] the cross-sectional study design, a convenience sample with an unknown degree of selection bias due to public recruitment, self-reported COVID-19 test results, the study population being largely White and healthy, and lack of information on breakthrough infections. Participants were given only 1 month to complete antibody testing, which may have contributed to the 52% rate among those invited to test.

Although evidence of natural immunity in unvaccinated healthy US adults up to 20 months after confirmed COVID-19 infection is encouraging, it is unclear how these antibody levels correlate with protection against future SARS-CoV-2 infections, particularly with emerging variants. The public health implications and long-term understanding of these findings merit further consideration.

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Durability of Anti-Spike Antibodies in Infants After Maternal COVID-19 Vaccination or Natural Infection

COVID-19 vaccination in pregnancy generates functional anti-spike (anti-S) IgG antibodies in maternal circulation that are detectable in umbilical cord blood at birth and can protect newborns and infants from COVID-19.[1-4] Anti-S IgG titers in the umbilical cord are correlated with maternal titers and are highest after late second and early third trimester vaccination.[2-4] We characterized the persistence of vaccine-induced maternal anti-S IgG in infant blood and compared persistence of infant anti-S IgG after maternal vaccination vs natural infection.

Supplemental content

Methods | The study included individuals who had received an mRNA COVID-19 vaccine in pregnancy or were infected with SARS-CoV-2 at 20 to 32 weeks' gestation, had enrolled in a prospective study at 2 academic medical centers in Boston, and had enrolled their infants in this follow-up study conducted from July 21, 2021, to October 22, 2021. Individuals vaccinated or infected at 20 to 32 weeks' gestation were enrolled because previous studies have demonstrated superior transplacental transfer of antibodies during this window compared with vaccination closer to delivery.[4,5] Those infected before vaccination were excluded. Matched maternal and umbilical cord serum samples were collected at birth. Infant capillary serum samples were collected via microneedle device at 2 months after birth for infants of vaccinated mothers and at 6 months for infants of mothers who were vaccinated and mothers who had been infected with SARS-CoV-2. Antibody titers against the SARS-CoV-2 spike protein were quantified using an enzyme-linked immunosorbent assay (eMethods in the Supplement). Differences in titers between vaccinated and infected groups at delivery and 6-month infant age were assessed by the Mann-Whitney *U* test. Differences in proportions of infants with detectable antibodies at 6 months were assessed by the Fisher exact test. Correlation between delivery titers and infant antibody was assessed via the Spearman rank test. Analyses were conducted using Prism version 9.0. Significance was defined as a 2-sided $P < .05$. The study was approved by the Mass General Brigham institutional review board, and all participants provided written informed consent.

Results | Seventy-seven vaccinated pregnant mothers and 12 with symptomatic SARS-CoV-2 infection in pregnancy were included (**Table**). At 2 months, capillary serum samples were collected from 49 infants of vaccinated mothers; at 6 months, serum samples were collected from 28 infants of vaccinated mothers (mean, 170 days after birth) and 12 infants of infected mothers (mean, 207 days after birth).

Vaccinated mothers had significantly higher titers at delivery, with a mean of 2.03 (SD, 0.47) optical density ($OD_{450\text{-}570}$), compared with mothers after infection, with a mean of 0.65 (SD, 0.76) $OD_{450\text{-}570}$ ($P < .001$). Similarly, the respective mean cord titers were higher after vaccination vs natural infection: 2.17 (SD, 0.50) $OD_{450\text{-}570}$ vs 1.00 (SD, 0.83) $OD_{450\text{-}570}$ ($P < .001$) (**Figure**). Among infants of vaccinated mothers at 2 months, 98% (48 of 49) had detectable anti-S IgG. The mean titer at 2 months was 1.29 (SD, 0.53) $OD_{450\text{-}570}$, which was correlated with both maternal ($r = 0.55$; $P < .001$) and cord titers ($r = 0.43$; $P = .01$) at delivery.

Vaccination resulted in significantly greater antibody persistence in infants than infection. At 6 months, 57% (16 of 28) of infants born to vaccinated mothers had detectable antibodies (Table) compared with 8% (1 of 12) of infants born to infected mothers ($P = .005$). Titers were a mean of 0.33 (SD, 0.46) $OD_{450\text{-}570}$ among infants of vaccinated mothers and 0 (SD, 0.01) $OD_{450\text{-}570}$ among infants of infected mothers ($P = .004$) (Figure). Neither maternal ($P = .23$) nor cord ($P = .05$) titers were significantly correlated with infant anti-S titers at 6 months, largely because 43% of infants had no detectable titer at that time.

Discussion | This study found that the majority of infants born to COVID-vaccinated mothers had persistent anti-S antibodies at 6 months compared with infants born to mothers with SARS-CoV-2 infection. Understanding the persistence of