Association between SARS-CoV-2 Symptoms, Ct Values, and Serological Response in Vaccinated and Unvaccinated Healthcare Personnel

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Background: SARS-CoV-2 vaccines are effective at reducing symptomatic and asymptomatic COVID-19. Limited studies have compared symptoms, threshold cycle (Ct) values from reverse transcription (RT)-PCR testing, and serological testing results between previously vaccinated vs unvaccinated populations with SARS-CoV-2 infection.
Methods: Healthcare personnel (HCP) with a positive SARS-CoV-2 RT-PCR test within the previous 14 to 28 days completed surveys including questions about demographics, medical conditions, social factors, and symptoms of COVID-19. Ct values were observed, and serological testing was performed for anti-nucleocapsid (anti-N) and anti-Spike (anti-S) antibodies at enrollment and 40 to 90 days later. Serological results were compared to HCP with no known SARS-CoV-2 infection and negative anti-N testing.
Results: There were 104 unvaccinated/not fully vaccinated and 77 vaccinated HCP with 2 doses of an mRNA vaccine at time of infection. No differences in type or duration of symptoms were reported ($P = 0.45$). The median (interquartile range [IQR]) Ct was 21.4 (17.6–24.6) and 21.5 (18.1–24.6) for the unvaccinated and vaccinated HCP, respectively. Higher anti-N IgG was observed in unvaccinated HCP (5.08 S/CO, 3.08–6.92) than vaccinated (3.61 signal to cutoff ratio [S/CO], 2.16–5.05). Anti-S IgG was highest among vaccinated HCP with infection (34 285 aribitrary units [AU]/mL, 17 672–61 775), followed by vaccinated HCP with no prior infection (1452 AU/mL, 791–2943), then unvaccinated HCP with infection (829 AU/mL, 290–1555). Anti-S IgG decreased 1.56% (0.9%–1.79%) per day in unvaccinated and 0.38% (0.03%–0.94%) in vaccinated HCP.
Conclusions: Vaccinated HCP infected with SARS-CoV-2 reported comparable symptoms and had similar Ct values relative to unvaccinated. However, vaccinated HCP had increased and prolonged anti-S and decreased anti-N response relative to unvaccinated.

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IMPACT STATEMENT

This study compared symptoms, SARS-CoV-2 RT-PCR Ct values, and serological response in healthcare professionals with COVID-19 who were vaccinated vs unvaccinated for SARS-CoV-2. Results indicate that, for healthy individuals, COVID-19 symptomology may be similar for those who have vs have not been vaccinated, that Ct values may not be useful for predicting infection severity or serological response, and that serological protection against future infection may be influenced by repeated exposure to SARS-CoV-2, either through infection or vaccination. This contributes to knowledge concerning the relationship between 3 measures of illness, as well as the impact of vaccination status on these measures.

INTRODUCTION

The mRNA vaccines BNT162b2 and mRNA-1273 are highly effective at reducing symptomatic COVID-19, asymptomatic infections, and adverse outcomes including hospitalization and intensive care unit admission (1–5). While the efficacy of these vaccines has been affected by the emergence of novel variants (6–8) and wanes with time from vaccination (9), there remains clear clinical benefit from vaccination for reducing the risk of infection in the weeks to months following immunization (10) and for decreasing the risk of severe disease or death. These effects have also been observed in healthcare personnel (HCP), who may experience a dramatic reduction in the rate of COVID-19 infection post-vaccination (11, 12).

While the effectiveness of mRNA vaccines in reducing SARS-CoV-2 infections and adverse outcomes has been clearly demonstrated in the literature, it is less clear whether vaccination affects the symptomatology associated with COVID-19. Further, while the rate of post-acute sequelae of SARS-CoV-2 infection (long COVID) is reduced in vaccinated populations, studies to date are inconclusive regarding the rate of long COVID in those that have infection after vaccination (13).

Interestingly, studies associating SARS-CoV-2 reverse transcription (RT)-PCR threshold cycle (Ct) values with clinical outcomes have demonstrated mixed results (14–17). Retrospective observational studies have implied a reduced viral load in vaccinated adults in the general population (18). However, the impact of vaccination on viral load in otherwise healthy populations with mild symptoms is unknown.

Protection provided by mRNA vaccines is due in part to the robust humoral immune responses generated to the SARS-CoV-2 spike (S) protein and remarkably high neutralizing antibody levels (19, 20). However, SARS-CoV-2-specific antibody levels are known to wane over time, particularly in individuals older than 65 years of age (21). Previous studies have demonstrated that individuals who have a SARS-CoV-2 infection after vaccination and those who are vaccinated after a previous infection have a particularly robust antibody response and protection against SARS-CoV-2 variants relative to those who receive vaccine only (22, 23). However, few studies have compared the serological response in individuals who are vaccinated after having a SARS-CoV-2 infection vs individuals who have a SARS-CoV-2 infection after being vaccinated. Further, little is known about how the serological response correlates with symptomatology and Ct values in vaccinated relative to unvaccinated individuals.

In this prospective observational study, we compared self-reported symptoms in vaccinated vs unvaccinated SARS-CoV-2 infected HCP and

associated these with Ct values and longitudinal serological testing.

MATERIALS AND METHODS

Setting and Study Population

This study took place at a large academic medical center in St. Louis, MO. HCP ≥18 years of age employed at Washington University School of Medicine (WUSM), Barnes-Jewish Hospital (BJH), or St. Louis Children's Hospital (SLCH) who had a positive SARS-CoV-2 PCR test from a nasopharyngeal (NP) swab within the previous 14 to 28 days were eligible to participate. Potential participants were identified using occupational health records and study advertisements. The unvaccinated group also included participants who were not fully vaccinated (i.e., only a single dose of vaccine received) or less than 14 days from the second dose of an mRNA vaccine at the time of enrollment. Some participants were vaccinated/became fully vaccinated after initial infection and entry into the study. These individuals were grouped in the "unvaccinated" category for both the entry and follow-up study visits.

To provide a comparison group, an additional 127 HCP who had no documented or reported RT-PCR-confirmed SARS-CoV-2 infection and who had received 2 doses of an mRNA vaccine were also recruited. Control HCP were included in the study only if they were negative for anti-SARS-CoV-2 nucleocapsid (anti-N) antibodies at enrollment.

The study protocol was reviewed and approved by the Washington University in St. Louis Human Research Protection Office (IRB# 202008054, 202010117). All participants provided informed consent prior to specimen and data collection.

Study Visits

Enrollment visits were conducted 14 to 28 days after the positive SARS-CoV-2 PCR test. Follow-up visits were conducted 40 to 90 days after the positive SARS-CoV-2 PCR test. All participants were enrolled between September 29, 2020, and February 15, 2022, and follow-up visits were conducted between December 8, 2020, and March 15, 2022. Vaccines were available to HCP beginning December 14, 2020. At each study visit, participants completed a survey that included questions about demographics, employment, pre-existing medical conditions, SARS-CoV-2 exposures, social distancing behaviors, travel and social event history, and symptoms of COVID-19. Pre-existing medical conditions screened included blood disorders, cancer, cerebrovascular disease, chronic kidney disease, diabetes, heart disease, history of solid organ transplant or bone marrow transplant, HIV, liver disease, lung disease, neurologic conditions, obesity, pregnancy, seasonal allergies, smoking status, and use of corticosteroids. Participants were also asked about their SARS-CoV-2 vaccine status, type of vaccine received, number of doses received, and date of each dose. In October 2021, a question was added to the follow-up visit survey about a third/booster dose but no participants had received a booster dose of vaccine at entry. Participants were considered fully vaccinated if they had received the second dose of mRNA vaccine at least 2 weeks prior to the relevant study visit.

Specimen Collection and Laboratory Testing

Blood specimens were collected into a 10 mL K_2 EDTA tube and maintained at room temperature for up to 8 h before refrigeration. Specimens were centrifuged and plasma aliquots stored for up to 4 days at 4°C prior to anti-N testing, or frozen at −80°C for up to 12 months then thawed and clarified by centrifugation prior to anti-S testing.

Anti-N testing was performed using the Abbott SARS-CoV-2 IgG assay according to the manufacturer's recommendations on an Abbott Architect i2000. The assay is qualitative but also reports a signal to cutoff ratio (S/CO) relative to the calibrator. Several studies have previously reported an

association between the S/CO and an increase in antibody response including neutralizing antibodies (24–26). A result of ≥1.4 S/CO is considered a positive result. Anti-S testing was performed using the Abbott SARS-CoV-2 IgG II assay according to the manufacturer's recommendations. This assay is semi-quantitative and reports in arbitrary units (AU)/mL. Plasma samples testing above the analytic measuring range (25 000 AU/mL) of the assay were diluted and retested to report a final result.

Molecular testing for SARS-CoV-2 was performed on multiple FDA-emergency use authorization (EUA) or cleared platforms at our institution during the study enrollment period. The vast majority of HCP screenings were performed using the Roche cobas SARS-CoV-2 method on the cobas 6800 system according to the manufacturer's instructions. Other platforms were used for testing due to availability of tests or when participants were tested at outside laboratories. For quantitative comparisons of Ct values, only HCP results tested on the Roche instrument from the ORF-1 target were used.

Data Analysis

Fisher exact and chi-square tests were used to examine associations between SARS-CoV-2 vaccine status and HCP characteristics, SARS-CoV-2 exposure history, and symptoms. Normality was assessed using the Shapiro–Wilk test with each group in the study being non-normally distributed ($P < 0.05$). The Mann–Whitney test was used to compare 2 groups and multiple comparisons were performed using a non-parametric (Kruskal-Wallis) one way ANOVA with Dunn multiple comparison test and multiplicity-adjusted *P*-values. Correlation was performed using Spearman rank correlation coefficient (*r*). For comparing serological results, HCP were further broken down to those who had SARS-CoV-2 infection after 2 doses of vaccine, those who had 2 doses of vaccine after SARS-CoV-2 infection, and those who received a booster dose (3 doses of a vaccine). Statistical analyses were performed in SAS version 9.4 and GraphPad Prism 9 with $P < 0.05$ considered statistically significant. All data and statistical analysis were stored/saved locally on a shared folder for future use and analysis.

RESULTS

Demographics

We enrolled 104 HCP who were unvaccinated (n = 90) or incompletely vaccinated (n = 14) at the time of SARS-CoV-2 infection and 77 were fully vaccinated with 2 doses of an mRNA vaccine (Table 1). Fourteen (13.5%) of the HCP in the unvaccinated group were partially vaccinated or <2 weeks from their second dose of mRNA vaccine. Among vaccinated HCP, the median time from the second dose of vaccine to RT-PCR-confirmed SARS-CoV-2 infection was 267 days (range 17 to 435 days). The median (interquartile range [IQR]) age was comparable in the unvaccinated (38, 30–46) and vaccinated (38, 29–48) cohorts. Both cohorts were primarily white (unvaccinated 86.5% vs vaccinated 85.7%, $P = 0.87$) and primarily female sex (unvaccinated 86.5% vs vaccinated 76.6%, $P = 0.11$). The only common comorbidities that were reported by more than 10% of the study population were seasonal allergies and obesity, but there was no difference between unvaccinated and vaccinated cohorts. Similar rates of known recent occupational exposure were reported in the unvaccinated (14.6%) and the vaccinated (18.2%, $P = 0.51$) cohorts. Further, contact with COVID-19 patients (11.5% vs 11.7%, $P = 0.98$), adherence to social distancing measures more than half the time (66.3% vs 62.3%, $P = 0.52$), and wearing a face mask more than half the time while at work (98% vs 98.7%, $P = 1.0$) were comparable between the unvaccinated and vaccinated cohorts, respectively. Unvaccinated HCP reported lower rates of travel (12.5% vs 27.3%), attending gatherings such as a restaurant

Table 1. Healthcare personnel baseline demographics and risk behaviors.

	Vaccinated HCP n = 77 (%)	Unvaccinated HCP n = 104 (%)	*P*-value
Demographics			
Age (median, [IQR])	38 [30–46]	38 [29–47.5]	0.39
Female	59 (76.6)	90 (86.5)	0.11
Race			
Asian	6 (7.8)	4 (3.8)	0.33
Black	6 (7.8)	9 (8.6)	0.83
White	66 (85.7)	90 (86.5)	0.87
Other[a]	4 (5.2)	2 (1.9)	0.40
Hispanic ethnicity	2 (2.6)	5 (4.8)	0.70
Job role[b]			0.60
Patient care role[c]	52 (67.5)	71 (68.3)	
Patient support role[d]	25 (32.5)	32 (30.8)	
Comorbidities			
Seasonal allergies	29 (37.7)	47 (45.2)	0.31
Body mass index of 30 or higher	12 (15.6)	23 (22.1)	0.27
Cerebrovascular disease	7 (9.1)	4 (3.9)	0.21
Other[e]	13 (16.9)	30 (28.9)	0.06
Occupational risk factors			
Known, specific COVID-19 exposure at work[b]	14 (18.2)	15 (14.6)	0.51
Contact with known or suspected COVID-19 patients at work more than half the time[f]	9 (11.7)	12 (11.5)	0.98
Practice social distancing more than half the time when at work and not involved in immediate patient care[b]	48 (62.3)	69 (66.3)	0.52
Wear a face mask more than half the time while at work[b]	76 (98.7)	102 (98.0)	1.00
Non-occupational risk factors			
Household member suspected or confirmed to have COVID-19 in past 30 days	47 (61.0)	60 (57.7)	0.65
Known, specific COVID-19 exposure outside of work (excluding sick household member)	15 (19.5)	25 (24.0)	0.44
Practice social distancing more than half the time when in public[g]	66 (85.7)	101 (97.1)	0.009
Wear a face mask more than half the time while in public	70 (90.9)	103 (99.0)	0.011
Traveled in the past 30 days	21 (27.3)	13 (12.5)	0.012
Attended a gathering in the past 30 days			
Restaurant	46 (59.7)	31 (29.8)	<0.001
Other large gathering[h]	46 (59.7)	34 (32.7)	<0.001
None	18 (23.4)	49 (47.1)	0.001
Visited a public location in the past 30 days			
Medical office	29 (37.7)	25 (24.0)	0.048
Store	74 (96.1)	97 (93.3)	0.52
Other[i]	34 (44.2)	32 (30.8)	0.06

(continued)

Table 1. Continued

	Vaccinated HCP n = 77 (%)	Unvaccinated HCP n = 104 (%)	*P*-value
None	2 (2.6)	4 (3.8)	1.00

[a]Other race included Middle Eastern and mixed race.
[b]One HCP in the unvaccinated group was missing a response to this question.
[c]Patient care roles include: advance practice nurse, dialysis technician, medical assistant, medical student, nurse, nurse's assistant, nurse practitioner, occupational therapist, paramedic, patient care technician, patient sitter, perfusionist, physical therapist, physician, physician's assistant, psychologist, radiation technician, respiratory therapist, x-ray technician.
[d]Patient support roles include: administration, dietician, dining services personnel, facilities personnel, graduate student, laboratory personnel, pharmacist, pharmacy technician, postdoctoral fellow, research personnel, social worker.
[e]Other comorbidities include: asthma, autoimmune condition, blood disorder, cancer, Crohn disease, diabetes, hearing loss, heart disease, hyperthyroidism, hypothyroidism, liver disease, lung disease, migraines, neurologic condition, pregnancy, smoking, use of immunosuppressive medications.
[f]Two HCP in the unvaccinated group were missing a response to this question.
[g]One HCP in the vaccinated group was missing a response to this question.
[h]Other large gatherings include: amusement park, bar, concert, funeral, gathering of family or friends, graduation, religious service, school, sporting events, wedding.
[i]Other public locations include: bank, Department of Motor Vehicles, dry cleaner, hair salon, laundromat, library, post office, veterinarian office.

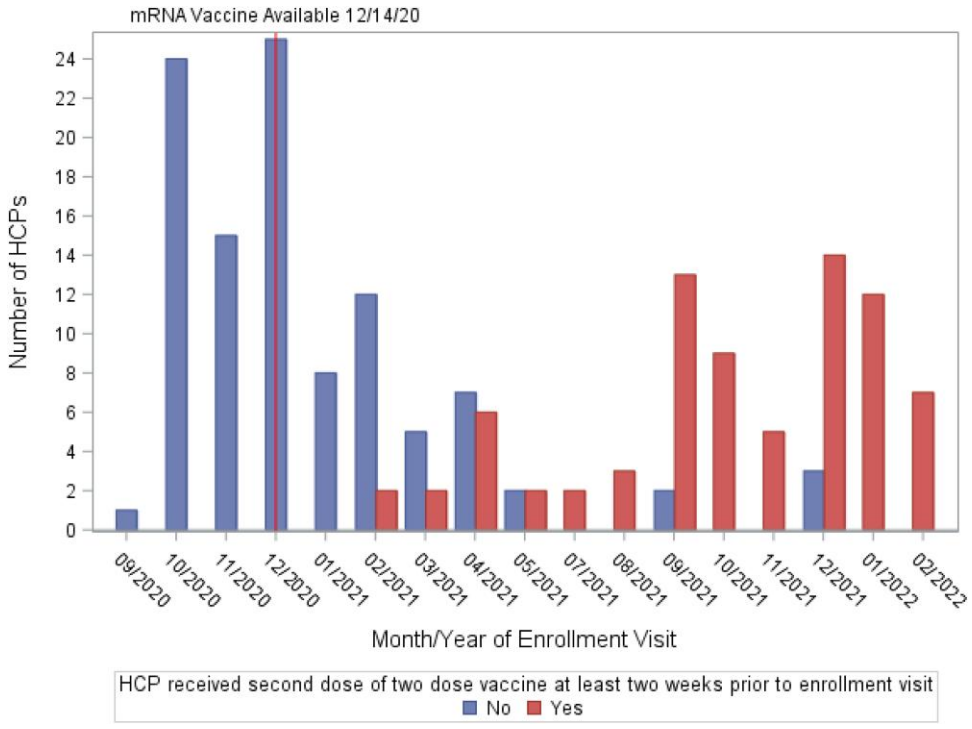

Fig. 1. Participant enrollment dates, vaccination status, and local availability of SARS-CoV-2 vaccines and boosters.
Each column is the number of individuals recruited in each month. Unvaccinated HCP are represented by the blue column and vaccinated by the red.

Table 2. Association between SARS-CoV-2 testing, symptomatology, and vaccination status in infected HCP.

	Vaccinated HCP n = 77 (%)	Unvaccinated HCP n = 104 (%)	*P*-value
SARS-CoV-2 Testing			
Days from positive SARS-CoV-2 PCR test to enrollment survey (median, [IQR])	23 [19–27]	23.5 [20–25]	0.75
Symptoms in past 14 days			
Any symptom	50 (64.9)	73 (70.2)	0.45
Fatigue	38 (49.3)	53 (51.0)	0.83
Congestion or runny nose	34 (44.2)	36 (34.6)	0.19
Headache	30 (39.0)	50 (48.1)	0.22
Cough	29 (37.7)	47 (45.2)	0.31
New loss of sense of taste or smell	26 (33.8)	31 (29.8)	0.57
Muscle or body aches	20 (26.0)	30 (28.8)	0.67
Fever or chills	11 (14.3)	18 (17.3)	0.58
GI symptoms	11 (14.3)	17 (16.3)	0.70
Sore throat	11 (14.3)	16 (15.4)	0.84
Shortness of breath	10 (13.0)	25 (24.0)	0.06
Other	2 (2.6)	6 (5.8)	0.47
More than one symptom	42 (54.5)	61 (58.6)	0.58
Ongoing symptoms at time of enrollment[a]			0.27
Yes	11 (14.3)	25 (24.0)	
No	38 (49.3)	48 (46.2)	
Never experienced symptoms	27 (35.1)	31 (29.8)	
Sought medical care for symptoms			0.74
Sought medical care	13 (16.9)	20 (19.2)	
Did not seek medical care	37 (48.0)	53 (51.0)	
Never experienced symptoms	27 (35.1)	31 (29.8)	

[a]One HCP in the vaccinated group was missing a response to this question.

(29.8% vs 59.7%), and visiting public locations such as a medical office (24.0% vs 37.7%) compared to vaccinated HCP ($P < 0.05$ for each). However, this difference likely reflected lockdown measures and early attempts to reduce the spread of COVID-19, as most unvaccinated individuals were enrolled between September 2020 and January 2021, before vaccines were widely available to HCP and when lockdown measures were more common in the region (Fig. 1).

Symptoms

No significant differences in symptomatology were reported in unvaccinated vs vaccinated HCP at study enrollment (Table 2). The most common symptoms for both groups were fatigue (51.0% for unvaccinated HCP vs 49.3% for vaccinated HCP) and congestion (34.6% for unvaccinated HCP vs 44.2% for vaccinated HCP). Approximately one third (29.8% of unvaccinated HCP and 35.1% of vaccinated HCP) reported no

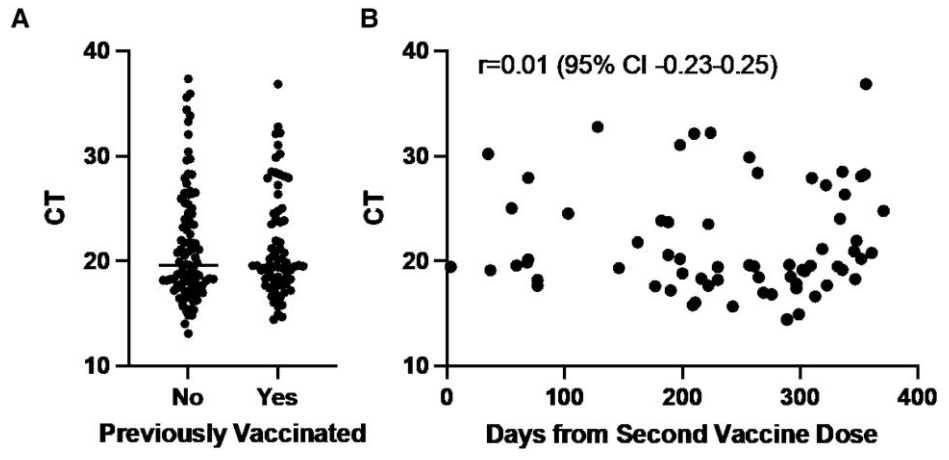

Fig. 2. Ct values in vaccinated and unvaccinated healthcare personnel.
Each dot is an individual participant. Previously vaccinated healthcare personnel were at least 14 days from their second dose. (A), Ct relative to vaccination status and (B), Ct values in vaccinated workers in time from second vaccine dose.

symptoms of COVID-19, despite having had a positive PCR test. A higher proportion of unvaccinated (24.0%) than vaccinated (13.0%) HCP reported shortness of breath, but this did not reach statistical significance ($P = 0.06$). At follow-up, there was no significant difference in duration of reported symptoms ($P = 0.37$) with 39.3% of unvaccinated and 49.3% of vaccinated HCP reporting that symptoms subsided within 14 days (online Supplemental Table 1). At the time of follow-up (range 45 to 140 days), 25.8% of unvaccinated and 19.0% of vaccinated HCP reporting ongoing symptoms. Of the HCP that were unvaccinated at entry, 26/104 (25%) were fully vaccinated at the follow-up visit.

Ct Values

For their initial positive PCR testing, 95/104 unvaccinated and 70/77 vaccinated HCP were tested for SARS-CoV-2 using the Roche RT-PCR assay. The mean (IQR) Ct value was 21.4 (17.6–24.6) for the unvaccinated group and 21.5 (18.1–24.6) for the vaccinated group (Fig. 2, A). In the vaccinated cohort, there was no association between time from second vaccine dose and Ct value (Pearson $r = 0.01$, Fig. 2, B). Independent of vaccination status, there was no difference in Ct in those with a body mass index (BMI) ≥30, those who were asymptomatic, those with persistent symptoms at follow-up, those who presented with fever, or those who presented with shortness of breath (online Supplemental Fig. 1).

Antibody Titers

At enrollment, HCP who were not vaccinated had higher anti-N antibody levels (median = 5.08 S/CO, IQR 3.08–6.92) than those who were vaccinated (median = 3.61, IQR 2.16–5.05, $P < 0.001$, Fig. 3, A). There was a significant decrease in anti-N antibodies in all participants at follow-up, but anti-N antibodies remained higher in unvaccinated HCP (median 2.83, IQR 1.51–5.46) than vaccinated HCP (median 1.70, IQR 0.93–2.81, $P < 0.001$, Fig. 3, B). There was no significant difference in Ct values between HCP who were anti-N positive and those who were anti-N negative, independent of vaccination status at enrollment or at follow-up (Fig. 3, C and D). Further, there was no significant

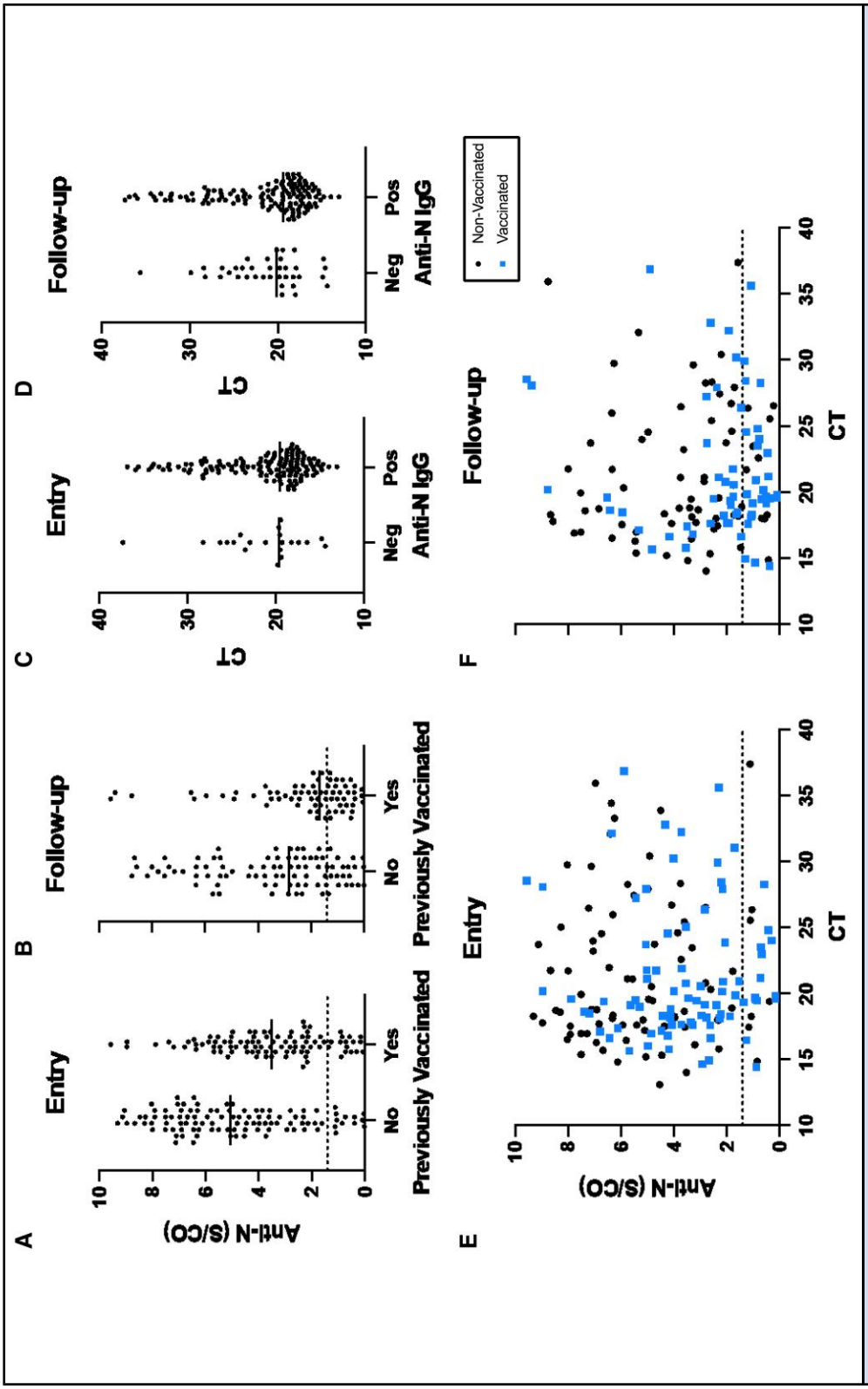

Fig. 3. Association between serologic response to SARS-CoV-2 infection and Ct values in vaccinated and unvaccinated healthcare personnel. (A), Serological response was assessed at 14 to 28 days and (B), 30 to 90 days after positive PCR; (C), Ct-stratified serological positive and negative at enrollment and (D), follow-up; (E), Association between anti-N and Ct values at enrollment and (F), follow-up. Blue squares are vaccinated and black circles are unvaccinated HCP. Dotted lines are the qualitative cutoff for a positive result by the serological assay.

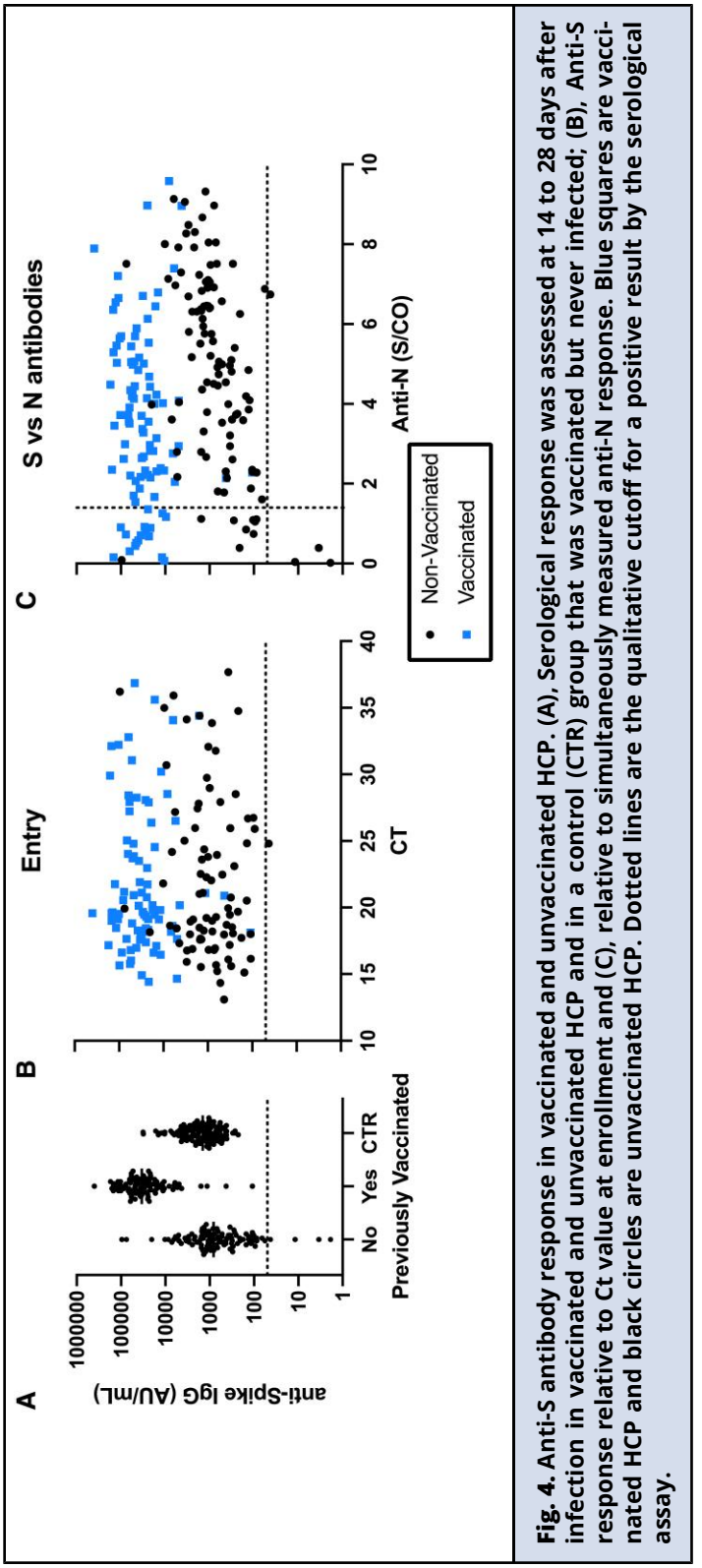

Fig. 4. Anti-S antibody response in vaccinated and unvaccinated HCP. (A), Serological response was assessed at 14 to 28 days after infection in vaccinated and unvaccinated HCP and in a control (CTR) group that was vaccinated but never infected; (B), Anti-S response relative to Ct value at enrollment and (C), relative to simultaneously measured anti-N response. Blue squares are vaccinated HCP and black circles are unvaccinated HCP. Dotted lines are the qualitative cutoff for a positive result by the serological assay.

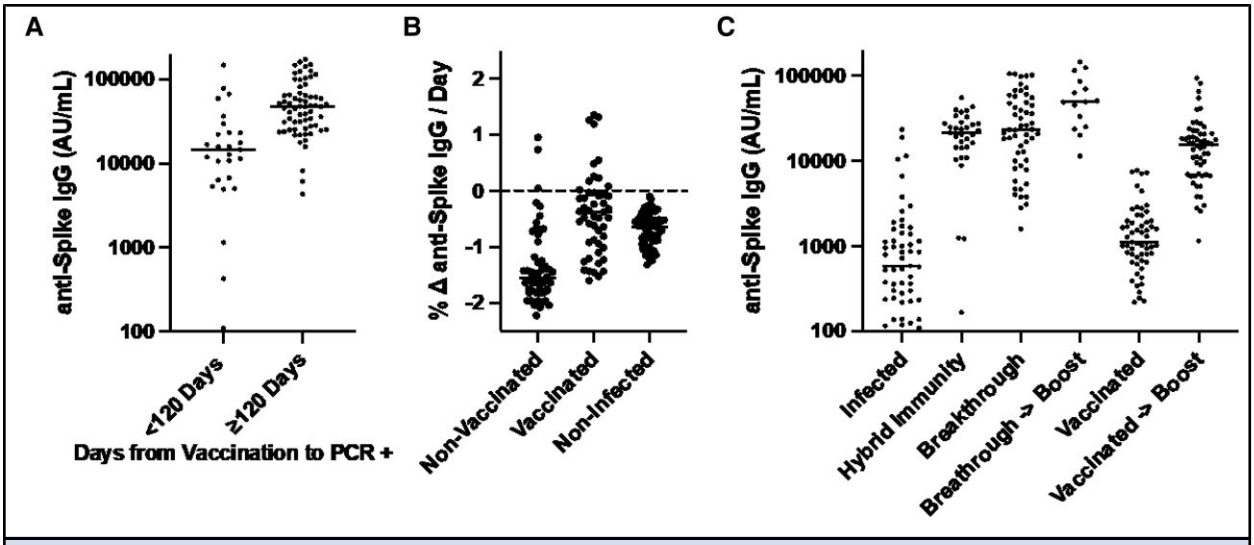

Fig. 5. Kinetics of anti-S antibody response. (A), Comparison of anti-S levels at entry in infected HCP <120 days and ≥120 days after vaccination; (B), Change in anti-S antibody levels over time in those with follow-up visits; (C), Anti-S levels in healthcare personnel at follow-up based on vaccination status and infection status. Infected = infected but not vaccinated (n = 55). Hybrid Immunity = vaccinated after infection (n = 33), Breakthrough = infection after vaccination (n = 54), Breakthrough -> Boost = infected after vaccination than receiving a third vaccination (n = 15), Vaccinated = fully vaccinated, non-infected (n = 58), Vaccinated -> Boost = 3 doses of vaccination (n = 56).

correlation between Ct value and anti-N antibodies at enrollment or at follow-up for the unvaccinated and the vaccinated groups ($P > 0.3$ for all, Fig. 3, E and F). There were no significant differences in anti-N antibody levels between obese (BMI ≥30) vs non-obese HCP, or between symptomatic and asymptomatic HCP, independent of vaccination status (online Supplemental Fig. 2). Comparison of Ct values, anti-N, and anti-S IgG for the unvaccinated and vaccinated HCP can be found in online Supplemental Table 2.

In the 14 to 28 day window following SARS-CoV-2 infection, unvaccinated HCP had lower anti-S antibody levels (median 829 AU/mL, IQR 290 to 1555) than HCP who were vaccinated prior to infection (median 34 285 AU/mL, IQR 17 672 to 61 775, $P < 0.001$) or vaccinated HCP with no prior infection (median 1452 AU/mL, IQR 791 to 2943, $P = 0.005$) (Fig. 4, A). There was no correlation between enrollment Ct value and anti-S IgG among vaccinated ($r = 0.03$, −0.19 to 0.25) or unvaccinated HCP ($r = 0.11$, −0.11 to 0.32) (Fig. 4, B). Anti-N IgG and anti-S IgG at enrollment were highly correlated in the unvaccinated ($r = 0.52$, 0.4 to 0.65, $P < 0.001$), but not in the vaccinated cohort ($r = 0.19$, −0.02 to 0.38, $P = 0.06$) (Fig. 4, C). As with anti-S antibody titers, no significant differences were observed in anti-N antibody levels in those with BMI ≥30 or in patient-reported symptoms, independent of vaccination status (online Supplemental Fig. 3).

HCP who were vaccinated <120 days prior to PCR-confirmed SARS-CoV-2 infection had a reduced median anti-S IgG response (14 689, IQR 6378–23 460) relative to those vaccinated ≥120 days prior to infection (47 491, IQR 26 164–68 750, $P < 0.001$) (Fig. 5, A). Among HCP with no change in vaccination status between visits, the median percent decrease in anti-S IgG per day was 1.56% (IQR 0.9%–1.79%) in unvaccinated, infected HCP; 0.38% (IQR 0.03%–0.94%) in vaccinated, infected HCP; and 0.64 (IQR 0.52%–0.89%) in vaccinated, non-infected HCP (Fig. 5, B). Thirty-three infected HCP who were

unvaccinated at enrollment were vaccinated prior to follow-up and 15 infected HCP who had breakthrough infections received a booster dose of mRNA vaccine prior to follow-up. Vaccinated HCP who had a breakthrough infection and then received a booster had the highest anti-S antibody titers at follow-up (49 614, IQR 25 218–86 466, Fig. 5, C). This was followed by vaccinated HCP with breakthrough infections who had not received a booster (23 275, IQR 9739–50 545), HCP who were vaccinated after infection (21 412, IQR 14 383–28 098), uninfected HCP who were vaccinated and had received a booster (15 416, IQR 6971–20 985), uninfected HCP with 2 doses of an mRNA vaccine (1110, IQR 728–1995), and unvaccinated HCP who remained unvaccinated at follow-up (589, IQR 236–1439).

DISCUSSION

Vaccines against SARS-CoV-2 have high efficacy, and original trials of the mRNA-1273 and the BNT162b2 vaccines demonstrated >90% efficacy at preventing COVID-19 illness (3, 4) and adverse clinical outcomes, including the need for invasive ventilation and death (5, 27, 28). However, limited data is available simultaneously comparing symptoms of COVID-19, Ct values from SARS-CoV-2 RT-PCR results, and serological response in relatively healthy vaccinated and unvaccinated individuals.

A major finding from this study was that similar symptoms were self-reported by vaccinated and unvaccinated HCP who had a positive SARS-CoV-2 PCR test. While SARS-CoV-2 vaccination reduces infection rates, improves outcomes, and reduces hospitalizations among the general public (1–5), minimal data is available in the literature assessing symptomatology among relatively healthy, low risk-populations. In our study, 75% of vaccinated HCP were >4 months from their second dose of vaccine, with 25% > 1 year. The waning antibody response demonstrated here and in previous studies (29, 30) may underlie the similarities in reported symptoms between vaccinated and unvaccinated HCP. Nonetheless, our findings are seemingly in conflict with those of Antonelli et al., who reported reduced frequency and duration of COVID-19-related symptoms in vaccinated individuals (31). It is important to note that the Antonelli study included at-risk individuals including the elderly (>60 years), while our study was limited to healthy, working adults. Together, these results demonstrate that symptomatology may be comparable in those that do not require hospitalization from SARS-CoV-2 infection.

Previous studies have demonstrated an association between decreased Ct values and worse outcomes in hospitalized patients implying that Ct values may be a useful surrogate for predicting infection severity (32). We observed no difference in Ct values between unvaccinated vs the vaccinated HCP. Further, we observed no difference in Ct values among symptomatic vs asymptomatic HCP, those that presented with fever or shortness of breath vs those that did not, or in HCP with persistent symptoms lasting several months following initial infection. There are several biological (i.e., viral dynamics) and pre-analytical factors (i.e., sufficiency of swab collection) that can cause variation in SARS-CoV-2 Ct values (33–35). Our findings suggest Ct values may not be a useful surrogate for infection severity in relatively healthy individuals; higher-powered studies are needed to confirm this finding.

We also found no association between Ct value and anti-N or anti-S antibody levels in vaccinated or unvaccinated/partially vaccinated HCP. The non-association between Ct value and antibody responses suggests that host factors other than viral kinetics are likely driving the immune response in otherwise healthy individuals. This is consistent with our previous observation that HLA genotype is associated with SARS-CoV-2 infection outcomes and suggests an important role for host-specific antigen presentation (36, 37).

Similarly previous studies have demonstrated an association between HLA-class II polymorphisms and antibody levels after SARS-CoV-2 vaccination and infection (38–40). Together, our findings suggested limited utility in using Ct from routine RT-PCR for predicting severe symptoms or association with future serological responses.

Previous studies have demonstrated that patients with breakthrough infections (SARS-CoV-2 infection after 2 doses of vaccine) and those who receive 2 doses of vaccine after SARS-CoV-2 infection have enhanced neutralizing antibody response and efficacy against SARS-CoV-2 variants relative to those who receive vaccine only (22, 23, 41, 42). Here we also observed a dramatic increase in anti-S IgG in participants who had a history of both SARS-CoV-2 vaccination and SARS-CoV-2 infection. Similarly, studies have demonstrated improved immunogenicity with a third dose of BNT162b2 vaccine relative to the second dose (43). This is consistent with our study findings of approximately 10-fold higher anti-S IgG titers among boosted vs non-boosted HCP. Unique to our study is the comparison of serological response across multiple iterations of exposure to SARS-CoV-2 antigens, including: infection without vaccination; vaccination after infection; infection after vaccination (both with and without a subsequent booster dose); and vaccination plus a booster with no history of infection. While our results are limited by variation in time between vaccination and infection, it is clear from our data that repeated exposure to SARS-CoV-2 antigens via vaccine or live virus leads to enhanced immunogenicity. In fact, we observed no difference in serological response among HCP who were vaccinated after being infected, HCP who were infected after being vaccinated, and HCP who were fully vaccinated and had received a booster vaccine. This may imply that serological protection from future infection and SARS-CoV-2 variant strains is a function of repeated exposure and the production of antibodies with increasing affinity (44).

This study demonstrated lower anti-N antibodies in previously vaccinated individuals relative to unvaccinated individuals after infection, an effect that persisted through the follow-up visit (40 to 90 days after the positive PCR test). This is comparable to results previously published by McGee et al. (45). This may be due to differing mechanics of antigen presentation of live virus in vaccinated individuals or priming of the immune response to preferentially respond to the S antigen (46). Of note, the N protein is one of the most abundantly expressed and antigenic SARS-CoV-2 proteins, exceeding S expression by about 10-fold (47, 48). While interesting, the clinical ramifications of this finding are unclear. However, previous studies have suggested that higher anti-N titers are associated with worse outcomes; primarily thought to be a function of increased inflammation (49). Nonetheless, the finding of reduced anti-N IgG in vaccinated individuals is interesting and requires further evaluation.

Our study had several imitations. Our cohort was small due to the relative difficulty of recruiting recently infected HCP and the need to obtain blood at 2 separate visits (and resulting loss to follow-up). In addition, the cohort was composed primarily of white females, which reflected the racial and gender composition of HCPs employed at our facility. Both symptoms and vaccination data in this study were self-reported, and may be subject to recall bias. This study spanned several years, during which time multiple SARS-CoV-2 variants with potentially differing symptomatology were circulating (50). Furthermore, how infection with different SARS-CoV-2 variants impacts the serological response or viral load has not been well documented and may underlie differences observed here. Finally, recruitment for this study preceded the emergence of the Omicron variant. Mutations in the spike protein of Omicron and other emerging variants of concern have been demonstrated to reduce

neutralizing potency of both vaccines and convalescent plasma (51).

CONCLUSION

Among HCP that are infected with SARS-COV-2, vaccinated HCP report comparable symptoms and have similar Ct values by RT-PCR to unvaccinated HCP. However, vaccinated HCP had increased and prolonged anti-S and decreased anti-N response relative to unvaccinated HCP. These results may have implications for future immunity and require further interrogation.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

Nonstandard Abbreviations: Ct, threshold cycle; RT-PCR, reverse transcriptase PCR; HCP, healthcare personnel; N, nucleocapsid protein; S, spike protein; IQR, interquartile range; S/CO, signal to cutoff.

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