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Research Paper

Comparison of IgG and neutralizing antibody responses after one or two doses of COVID-19 mRNA vaccine in previously infected and uninfected individuals.

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ABSTRACT

Background: Recent reports have suggested that among individuals previously infected with SARS-CoV-2, a single mRNA vaccine dose is sufficient to elicit high levels of immunity.
Methods: We compared anti-SARS-CoV-2 spike receptor binding domain (RBD) IgG antibody concentrations and antibody-mediated neutralization of spike-angiotensin-converting enzyme (ACE2) receptor binding *in vitro* following vaccination of non-hospitalized participants by sero-status and acute virus diagnosis history. Participants were analysed before and after mRNA vaccination (BNT162b2/Pfizer or mRNA-1273/Moderna) in a community-based, home-collected, longitudinal serosurvey in the Chicago area (USA); none reported hospitalization for COVID-19. Samples were collected in January and February 2021. Before vaccination, some reported prior positive acute viral diagnostic testing and were seropositive (COVID-19+); the others who did not report acute viral diagnostic testing were categorized as seropositive or seronegative based on anti-spike RBD IgG test results.
Findings: Of 307 unique vaccine recipients, 46 reported a prior COVID-19 diagnosis and were seropositive (COVID-19 +). Of the 261 with no history of acute viral diagnostic testing, 117 were seropositive and 144 seronegative before vaccination. The median age was 38 years (range 21–83) with 67 female and 33% male; 40% were non-White. Responses were evaluated after one (n = 142) or two (n = 191) doses of BNT162b2 or mRNA-1273 vaccine. After one dose, median post-vaccine IgG concentration and percent surrogate neutralization were each significantly higher among the COVID-19+ (median 48·2 μg/ml, IgG; > 99.9% neutralization) compared to the seropositives (3·6 μg /ml IgG; 56.5% neutralization) and seronegatives (2·6 μg /ml IgG; 38·3% neutralization). The latter two groups reached > 95% neutralization after the second vaccine dose.
Interpretation: After one dose of mRNA vaccine, individuals previously diagnosed with COVID-19 responded with high levels of anti-RBD IgG and surrogate neutralization of spike-ACE2 interaction. One dose of mRNA vaccine was not sufficient to generate comparably high responses among most persons previously infected with SARS-CoV-2 without a clinical COVID-19 diagnosis, nor among seronegative persons.
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Research in context

Evidence before this study

Results from clinical trials have documented the effectiveness of two-dose mRNA vaccines in preventing symptomatic SARS-CoV-2 infections. Recent journal publications have indicated that a single dose of mRNA vaccine might be sufficient to boost immunity to high levels among previously infected individuals.

Added value of this study

We compared antibody response to vaccination across three groups: previously recovered from a confirmed outpatient case of COVID-19, seropositive for prior infection with SARS-CoV-2 but no acute virus diagnostic test for COVID-19, and seronegative for prior SARS-CoV-2 infection prior to vaccination. Since the majority of infections in the community are mild or asymptomatic, it is important to investigate whether patterns of vaccine response differ according to SARS-CoV-2 exposure history. We document robust responses to the first vaccine dose in the recovered COVID-19 group, but more heterogeneous and modest responses among seronegative individuals and seropositive individuals with mild/asymptomatic infections prior to vaccination.

Implications of all the available evidence

Our results suggest caution in assuming immunological priming and enhanced responses to a first mRNA vaccine dose among all individuals who are seropositive for prior SARS-CoV-2 infection. Similar to seronegative individuals, two doses are required for the seropositive group to attain a level of antibody response that approaches that of individuals with confirmed prior cases of symptomatic COVID-19.

1. Introduction

In December 2020, the FDA authorized the emergency use of two SARS-CoV-2 mRNA vaccines that utilize a 2-dose schedule: BNT162b2/Pfizer and mRNA-1273/Moderna [1,2]. While phase 3 trials for both vaccines reported high efficacy in preventing symptomatic SARS-CoV-2 infections after administration of the second dose, recent reports have suggested that a single dose is sufficient to boost immunity to high levels among previously infected individuals [3–6]. Since anti-SARS-CoV-2 seropositivity significantly exceeds documented COVID-19 cases in the community [7–9], it is important to investigate whether patterns of vaccine response differ according to SARS-CoV-2 exposure history. This is particularly important since the majority of infections are mild or asymptomatic, and severity of symptoms predicts the level of antibodies following natural infection [10,11].

We compared antibody response to vaccination across three groups: previously recovered from a confirmed outpatient case of COVID-19, seropositive for anti-SARS-CoV-2 receptor binding domain (RBD) IgG but no acute virus diagnostic test for COVID-19, and seronegative for prior SARS-CoV-2 infection prior to vaccination. Since the beginning of the pandemic, serological studies of the response to natural infection or vaccination have focused primarily on convalescent clinical cases or select populations of individuals for whom venous blood collection is relatively easy to implement (e.g., healthcare workers) [12,13]. Community-based serological studies can generate more diverse and representative samples, but are impeded by the logistical and technical challenges associated with venous blood collection and live virus neutralization methods. In this study, we use data from an ongoing community-based seroprevalence study [7,8] that combines in-home collection of finger stick dried blood spot (DBS) samples with the measurement of anti-RBD IgG, as well as the level of inhibition of interaction between the SARS-CoV-2 spike protein and the human angiotensin-converting enzyme (ACE2) receptor [14–16].

Finger stick DBS sampling is a minimally-invasive alternative to venipuncture that facilitates blood collection in non-clinical settings, using materials and methods that have been the foundation of nation-wide newborn screening programs since the 1960s [17–19]. An important advantage of DBS sampling—particularly in the context of the COVID-19 pandemic—is that it allows self-sampling in the home, and samples can be safely transported to the lab through the mail without a cold chain or special handling [19]. Quantification of anti-RBD IgG provides a measure of antibody response to an immunogenic glycoprotein on the viral surface that mediates attachment to host cells and facilitates viral entry [20–22]. Receptor blocking assays measure the ability of neutralizing antibodies to bind to viral proteins and inhibit entry into host cells [23]. For SARS-CoV-2, the surface spike protein engages the ACE2 receptor, and a surrogate virus neutralization assay provides an *in vitro* test of the effectiveness of anti-spike neutralizing antibodies in blocking this interaction [15,16]. In our community-based sample, we document robust responses to the first vaccine dose in the recovered COVID-19 group, but more heterogeneous and modest responses among those with no acute virus diagnostic testing who were either seropositive or seronegative. Results suggest caution in assuming immunological priming and enhanced responses to a first mRNA vaccine dose based on the presence of anti-SARS-CoV-2 antibodies alone.

2. Methods

Study approvals. Research activities were implemented under conditions of informed consent with protocols approved by the institutional review board at Northwestern University (#STU00212457, and #STU00212472).

Participants and study design. Approximately 8000 pre-vaccinated participants were recruited from the Chicago and area from April to December 2020. Eligible participants provided informed consent online and completed a questionnaire regarding COVID-19 status and symptoms. Participants received and returned materials for finger-stick DBS collection through the mail or on-site pick up. During this period, the seropositivity rate was 17·9% in the cohort [24]. Survey responses and anti-RBD IgG serology (described below) were used to categorize participants as recovered COVID-19 (positive clinical molecular diagnostic test for acute SARS-CoV-2 infection any time prior to vaccination), seropositive (no positive SARS-CoV-2 clinical diagnostic test result and positive for anti-RBD IgG), and seronegative (no positive SARS-CoV-2 clinical diagnostic test result and negative for anti-RBD IgG). These samples were used for the pre-vaccination serology.

From December 2020 through January 2021, SCAN participants were queried regarding COVID-19 vaccination status, and a subset reported receiving one or two doses of either mRNA vaccine and agreed to post-vaccination resampling. Participants were mailed a DBS kit (64 COVID-19, 167 pre-vaccination seropositive, and 160 pre-vaccination seronegative). Participants self-collected DBS at home through February 2021 and returned samples by mail. 307 unique participants returned samples. Twenty-six participants provided samples after both dose 1 and dose 2. We excluded DBS collected earlier than 9 days after dose 1 or earlier than day 5 after dose 2. Participants were categorized as recovered COVID-19 if they reported testing positive for SARS-CoV-2 on a clinical molecular diagnostic test for acute infection any time prior to vaccination.

Serological assays. The enzyme linked immunosorbent assay (ELISA) protocol to quantify anti-RBD IgG in DBS was previously

described, and is based on a widely used serum-based protocol with emergency use authorization [25,26]. Briefly, the day before the assay, plates were coated with spike RBD antigen (2 ug/mL PBS), and one 5 mm disc of each DBS sample was eluted in 250 uL PBS. The following day, the plate was washed and blocked, and 100 uL DBS eluate was transferred in duplicate and incubated for two hours. Anti-human IgG peroxidase was used as the detection antibody, and absorbance following the addition of chromogenic substrate was read at 490 nm (BioTek ELx808). A dilution series of CR3022, a monoclonal IgG with known reactivity to SARS-CoV-2 RBD, was used to generate a standard curve from which unknown concentrations were calculated based on four parameter logistic fit (Gen5, BioTek). For the anti-RBD IgG ELISA assay the inter-assay% coefficient of variation (%CV; standard deviation/mean x 100) for high (6·25 ug/mL), mid (1·56), and low (0·39) control samples included on each plate was 2·39, 9·20, and 5·99, 12·6 respectively. The intra-assay% coefficient of variation of duplicates across all samples was 3·7. Cross-reactivity of non SARS-CoV-2 antibodies (Varicella, Influenza, HSV, Rubella, Hepatitis, HIV, elevated IgG, elevated IGM, CMV) against RBD was not detected [27]. DBS samples were run in duplicate and reported as the average. A value $>$ 0·39 μg/ml CR3022 was considered positive based on prior analysis of confirmed negative (pre-pandemic) DBS samples [28]. Samples above the assay range were diluted, re-run, and the final result multiplied by the dilution factor.

The surrogate virus neutralization assay to quantify blocking of spike-ACE2 interaction was previously described [29], and is based on modifications to a commercially available protocol (Meso Scale Discovery V-PLEX SARS-CoV-2 Panel 2 Kit; K15386U-2). Briefly, DBS samples were eluted overnight at 4 °C in 100 uL assay diluent following removal of one 5.0 mm disc with a pneumatic hole punch (Analytical Sales and Services #327,500, Flanders, NJ). The following day, 25 uL of each sample were transferred in duplicate to a solid phase assay plate coated with SARS-CoV-2 spike antigen (wild type, Wuhan), followed by the addition of 25 uL of ACE2 conjugated with an electrochemiluminescent label. The plate was washed, read buffer was added to each well, and mean fluorescence intensity was read using a MESO® QuickPlex SQ 120 MM Imager. Percent neutralization was calculated as follows:% neutralization = 100 $\times$ 1-(sample MFI/negative control MFI). Three DBS control samples with high (mean = 96·5%), mid (76·0), and low (35·8) levels of neutralization were included on each assay plate. The inter-assay%CV for each control was 1·0, 9·1, and 15·0, respectively. Mean intra-assay%CV of duplicates across all samples was 3·3. Prior validation studies indicate that results from surrogate virus neutralization assays correlate highly with results from conventional live virus (Pearson R = 0·93) and pseudovirus neutralization methods (R = 0·92) [30]. In addition, results from matched DBS and serum samples indicate high agreement (concordance correlation = 0·991) in results for the surrogate virus neutralization protocol [14].

Statistical Analysis. Non-parametric K-sample equality of medians and Pearson chi-squared tests were used to evaluate differences across the COVID-19, seropositive, and seronegative groups. Descriptive statistics and two-way Mann-Whitney tests were used to evaluate differences in antibody concentration and% surrogate neutralization across groups. All analyses were implemented in Stata SE 15·1 (StataCorp, College Station, TX). $P < 0·05$ was considered statistically significant.

Role of funding source. The funding sources had no role in the study design, data collection, analysis, interpretation, or writing of the report. Funding sources do not have data access and were not involved in the decision to submit results for publication. AD and TM had full data access and took the decision to submit for publication.

3. Results

The study groups were comparable in terms of age, gender, and type of vaccine (all $p > 0·3$) (Table 1). Individuals who identify as Hispanic were over-represented in the COVID-19 group (X^2 = 16·3, $p < 0·05$). The recovered COVID-19+ group reported a median of five (interquartile range (IQR): 4–7) symptoms of COVID-19 infection and had a median interval of 138 days between their positive acute SARS-CoV-2 diagnostic test and vaccination. The seropositive group, with no prior diagnostic testing, reported a median of one symptom (IQR: 0–3), (z = 5·55, $p < 0·001$).

Prior to vaccination, median anti-RBD IgG concentration (z = 3·79, $p < 0·001$) and% surrogate neutralization (z = 5·14, $p < 0·001$) were significantly lower for seropositive participants in comparison with the recovered COVID-19-positive group. As expected, anti-RBD IgG (z = 13·83, $p < 0·001$) and surrogate neutralization (z = 2·66, $p < 0·01$) were significantly lower for seronegative participants in comparison with seropositives prior to vaccination (Fig. S1).

Following the first vaccine dose, there were large increases in anti-RBD IgG and surrogate neutralization for recovered COVID-19+ cases, and modest increases for the seropositive group (Fig. 1). In the COVID-19+ group, median IgG increased from baseline by a factor of 35·2, and all samples exceeded 95% neutralization. By contrast, in the seropositive group, anti-RBD IgG increased 5·6 fold and was significantly lower than the recovered COVID-19 positive group (z = 5·51, $p < 0·001$). Median surrogate neutralization was also significantly lower at 56·5% (z = 5·36, $p < 0·.001$), with only 4 of 46 samples reaching $>$ 95% neutralization. Post-dose 1 responses were higher for the seropositive in comparison with the seronegative group, but median IgG (3·6 vs. 2·6 ug/mL, z = 1·60, p = 0·11) and% neutralization (56·5 vs. 38·2, z = 1·54, p = 0·12) were not statistically significantly different. For the COVID-19 and seropositive groups, pre-vaccine anti-RBD IgG and% surrogate neutralization were positively associated with the magnitudes of response to the first vaccine dose (Fig. S2). For the seronegative group there was no association between the response to the first vaccine dose and pre-vaccine serology.

Following the second vaccine dose, the recovered COVID-19+ group had anti-RBD IgG and% neutralization that were comparable to the high levels recorded after the first dose. In the seropositive group, anti-RBD IgG was 7·1 times higher than after the first dose, but still significantly lower than the level observed in the COVID-19+ group (z = 3·23, $p < 0·01$). Percent neutralization was 1·7 times higher than after the first dose, but median neutralization remained significantly lower than the COVID-19+ group (z = 5·87, $p < 0·001$). Twenty four of 80 (30·0%) seropositive individuals did not exceed 95% neutralization after the second dose within our sampling timeframe. A comparable proportion (29·1%) of the seronegative group did not exceed 95% neutralization. The seropositive and seronegative groups did not differ significantly in anti-RBD IgG concentration (z = 0·70, p = 0·48) or% neutralization (z = 0·74, p = 0·46) after a second dose.

Anti-RBD IgG concentration positively correlated with% surrogate neutralization for all groups after one dose of vaccine, with a wide range of values for the seropositive and seronegative groups in comparison with the more consistently high levels among the recovered COVID-19+ group (Fig. 2).

In supplementary analyses we evaluated multivariate regression models predicting anti-IgG RBD and% surrogate neutralization after vaccine dose 1 and dose 2. Models included study group, as well as covariates listed in Table 1. Differences in dose 1 and dose 2 responses across groups were similar in bivariate and fully adjusted models, indicating that the pattern of results is not due to confounding by measured covariates.

4. Discussion

Following the initial phase of vaccine deployment, it has been suggested that two doses of currently available mRNA vaccines are not necessary for individuals previously infected with SARS-CoV-2 [3,5,6]. We document strong antibody responses to the first vaccine dose among individuals recovered from confirmed cases of COVID-

Table 1
Sample characteristics by pre-vaccine serology and timing of dried blood spot sample collection.

	COVID-19+			Seropositive			Seronegative			Total		
	Pre-Vaccine (*n* = 46)	Post-First Dose (*n* = 15)	Post-Second Dose (*n* = 32)	Pre-Vaccine (*n* = 117)	Post-First Dose (*n* = 46)	Post-Second Dose (*n* = 80)	Pre-Vaccine (*n* = 144)	Post-First Dose (*n* = 81)	Post-Second Dose (*n* = 79)	Pre-Vaccine (*n* = 307)	Post-First Dose (*n* = 142)	Post-Second Dose (*n* = 191)
Age *(Mdn, IQR)*	36.0 (27.0–43.0)	42.0 (32.0–57.0)	33.0 (27.0–41.0)	37.0 (29.0–50.0)	39.0 (30.0–51.0)	34.0 (28.0–45.5)	40.0 (31.0–53.0)	41.0 (31.0–63.0)	38.0 (30.0–46.0)	38.0 (30.0–50.0)	40.0 (31.0–60.0)	36.0 (28.0–45.0)
Birth sex *(n,%)*												
Male	14 (30.4)	4 (26.7)	10 (31.3)	40 (34.2)	15 (32.6)	30 (37.5)	46 (31.9)	27 (33.3)	27 (34.2)	100 (32.6)	46 (32.4)	67 (35.1)
Female	32 (69.6)	11 (73.3)	22 (68.8)	77 (65.8)	31 (67.4)	50 (62.5)	98 (68.1)	54 (66.7)	52 (65.8)	207 (67.4)	96 (67.6)	124 (64.9)
Race *(n,%)*												
Hispanic/Latinx	12 (26.1)	2 (13.3)	10 (31.3)	19 (16.2)	9 (19.6)	11 (13.8)	22 (15.3)	8 (9.9)	14 (17.7)	53 (17.3)	19 (13.4)	35 (18.3)
Non-Hispanic Asian	6 (13.0)	1 (6.7)	5 (15.6)	23 (19.7)	10 (21.7)	15 (18.8)	19 (13.2)	7 (8.6)	13 (16.5)	48 (15.6)	18 (12.7)	33 (17.3)
Non-Hispanic Black	1 (2.2)	0 (0.0)	1 (3.1)	6 (5.1)	3 (6.5)	3 (3.8)	2 (1.4)	2 (2.5)	0 (0.0)	9 (2.9)	5 (3.5)	4 (2.1)
Non-Hispanic White	24 (52.2)	11 (73.3)	14 (43.8)	61 (52.1)	21 (45.7)	45 (56.3)	99 (68.8)	62 (76.5)	52 (65.8)	184 (59.9)	94 (66.2)	111 (58.1)
Non-Hispanic Other	3 (6.5)	1 (6.7)	2 (6.3)	8 (6.8)	3 (6.5)	6 (7.5)	2 (1.4)	2 (2.5)	0 (0.0)	13 (4.2)	6 (4.2)	8 (4.2)
Vaccine manufacturer *(n,%)*												
BNT162b2/Pfizer	34 (73.9)	7 (46.7)	28 (87.5)	92 (78.6)	24 (52.2)	75 (93.8)	11 (77.1)	49 (60.5)	73 (92.4)	237 (77.2)	80 (56.3)	176 (92.2)
mRNA-1273/Moderna	12 (26.1)	8 (53.3)	4 (12.5)	25 (21.4)	22 (47.8)	5 (6.3)	32 (22.2)	31 (38.3)	6 (7.6)	69 (22.5)	61 (43.0)	15 (7.8)
Missing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	1 (1.2)	0 (0.0)	1 (0.3)	1 (0.7)	0 (0.0)
Immunoglobulin G (μg/ml) *(Mdn, IQR)*	1.4 (0.6–3.3)	48.2 (39.4–88.2)	46.8 (33.9–63.0)	0.6 (0.5–1.0)	3.6 (1.4–8.2)	25.5 (15.9–47.6)	0.1 (0.0–0.2)	2.6 (0.3–6.8)	22.4 (13.0–44.8)	0.4 (0.1–0.8)	3.6 (0.8–9.3)	28.7 (16.4–50.9)
Neutralizing antibody percent *(Mdn, IQR)*	21.8 (7.6–44.3)	99.9 (99.9–99.9)	99.9 (99.9–99.9)	4.4 (0.0–13.5)	56.5 (19.8–78.2)	98.4 (93.5–99.8)	2.2 (0.0–6.3)	38.3 (16.0–73.7)	97.9 (93.4–99.7)	3.9 (0.0–11.4)	53.7 (19.7–84.5)	99.1 (95.0–99.9)
DBS sample acquisition												
Days since most recent positive viral test *(Mdn, IQR)*	77.0 (45.5–172.5)[a]	119.0 (70.5–209.5)[b]	200.0 (158.0–312.5)[c]	-	-	-	-	-	-	-	-	-
Days since pre-vaccine sample *(Mdn, IQR)*	-	77.0 (28.0–92.0)	106.0 (97.0–129.5)	-	104.0 (92.0–135.0)	125.0 (106.0–159.0)	-	85.0 (74.0–173.0)	133.5 (81.0–193.0)	-	92.0 (75.0–166.0)	121.0 (93.0–169.0)
Days since receipt of first vaccine dose *(Mdn, IQR)*	-	17.0 (11.0–20.0)	44.0 (33.5–51.0)	-	18.5 (14.0–21.0)	45.0 (36.0–52.0)	-	18.0 (14.0–20.0)	40.0 (32.0–45.0)	-	18.0 (14.0–20.0)	42.0 (34.0–51.0)

Notes: Mdn=median; IQR=interquartile range.
[a] Date of most recent positive viral test was missing (*n* = 2). Date of most recent positive viral test occurred after sample collection (*n* = 4).
[b] Date of most recent positive viral test was missing (*n* = 1).
[c] Date of most recent positive viral test was missing (*n* = 1).

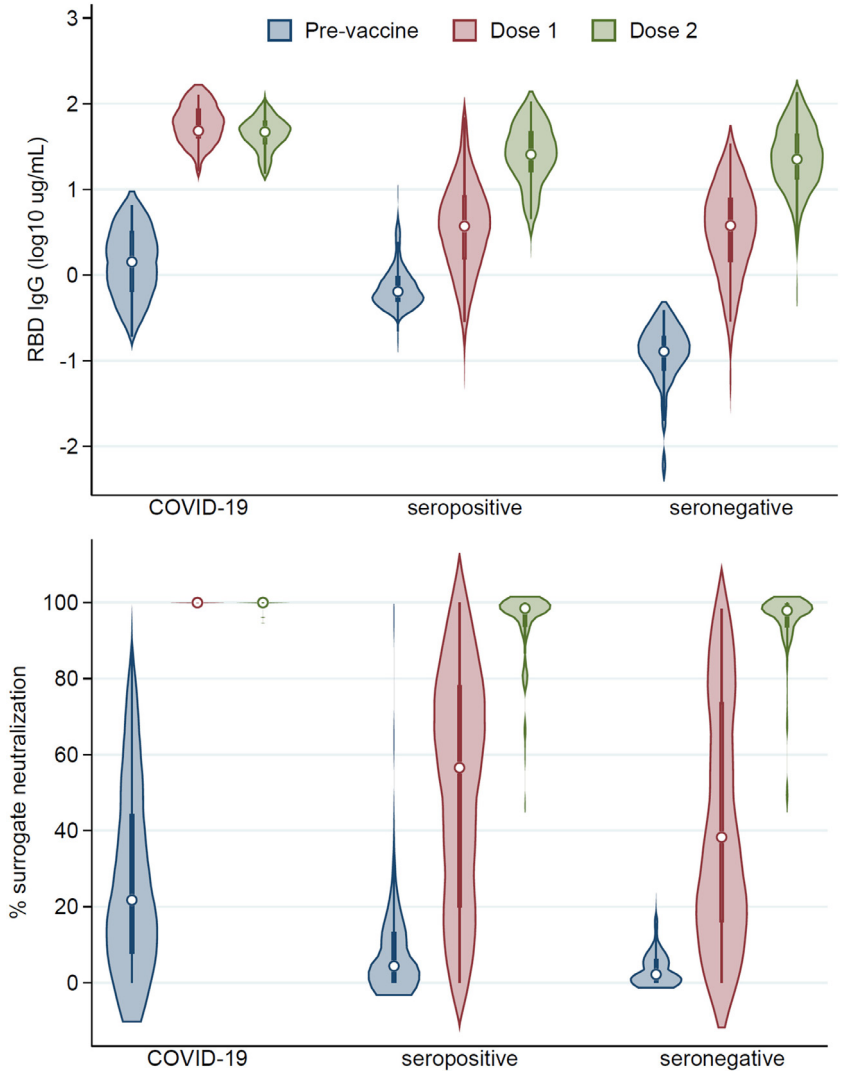

Fig. 1. Pattern of anti-RBD IgG and surrogate neutralization response to vaccination across study groups. Violin plots show median level and interquartile range, with kernel density, before vaccination, after dose 1, and after dose 2, for three groups: convalescent COVID-19, seropositive for SARS-CoV-2, and seronegative. The COVID-19 group had significantly higher pre-vaccine median IgG (1•4 vs. 0•6 ug/mL, $p < 0$•001) and median% neutralization (22•2 vs. 4•4, $p < 0$•001) in comparison with the seropositive group. The COVID-19 group had significantly higher post-dose 1 median IgG (48•2 vs. 3•6 ug/mL, $p < 0$•001) and median% neutralization (99•9 vs. 56•5, $p < 0$•001) in comparison with the seropositive group. Post-dose 1 responses were higher for the seropositive group in comparison with the seronegative group, but median IgG (3•6 vs. 2•6 ug/mL, $p = 0$•11) and% neutralization (56•5 vs. 38•2, $p = 0$•12) were not significantly different. The COVID-19 group had significantly higher post-dose 2 median IgG (46•8 vs. 25•5 ug/mL, $p<0$•01) and median% neutralization (99•9 vs. 98•5, $p<0$•001) in comparison with the seropositive group. The seropositive and seronegative groups did not differ significantly in post-dose 2 median IgG (25•5 vs. 22•4, $p = 0$•48) or median% neutralization (98•5 vs. 97•9, $p = 0$•46).

19, consistent with recent reports [5,6]. We document a pattern of mild and heterogeneous responses to the first dose among individuals previously unexposed to SARS-CoV-2, with more robust responses following the second dose, consistent with clinical trials data [2]. Importantly, responses in the seropositive group suggest that immunity following the first vaccine dose is significantly lower than the recovered COVID-19 group. And like the seronegative group, two doses are required for the seropositive group to attain a level of antibody response that approaches that of the COVID-19 group.

An advantage of our community-based design is that the cohort is comprised of a large and diverse sample of participants drawn from the general population of the Chicagoland area. However, a limitation is that the subsample is drawn from individuals who had priority access to vaccination beginning in December 2020, and is therefore not representative of the larger cohort. Furthermore, since this is an observational study we did not have precise control over the vaccination protocol and timing of blood sampling. An additional limitation is that within the seronegative group, a small number of participants could have had asymptomatic COVID-19 within the time period between pre-vaccination and post-vaccination DBS sampling. The other two groups would not be affected by this study design. Lastly, while our method for quantifying surrogate neutralization is relatively low cost and high throughput, the use of a single dilution of finger stick whole blood makes it difficult to compare across studies using live virus or pseudovirus methods for assessing neutralization.

The majority of cases of COVID-19 are asymptomatic or minimally symptomatic and require only home-based treatment [8,9], and our community-based design includes this important group. Studies of vaccine effectiveness that focus on convalescent patients who were hospitalized for more serious COVID-19, or samples enriched with people at higher risk of exposure and potentially repeated exposures (e.g., health care workers), may lead to overestimates of the level of priming immunity that do not generalize to the entire population of seropositive individuals with reduced exposure to SARS-CoV-2 antigens during natural infection. Results from this study, with clinically confirmed symptomatic as well as asymptomatic/mild cases of

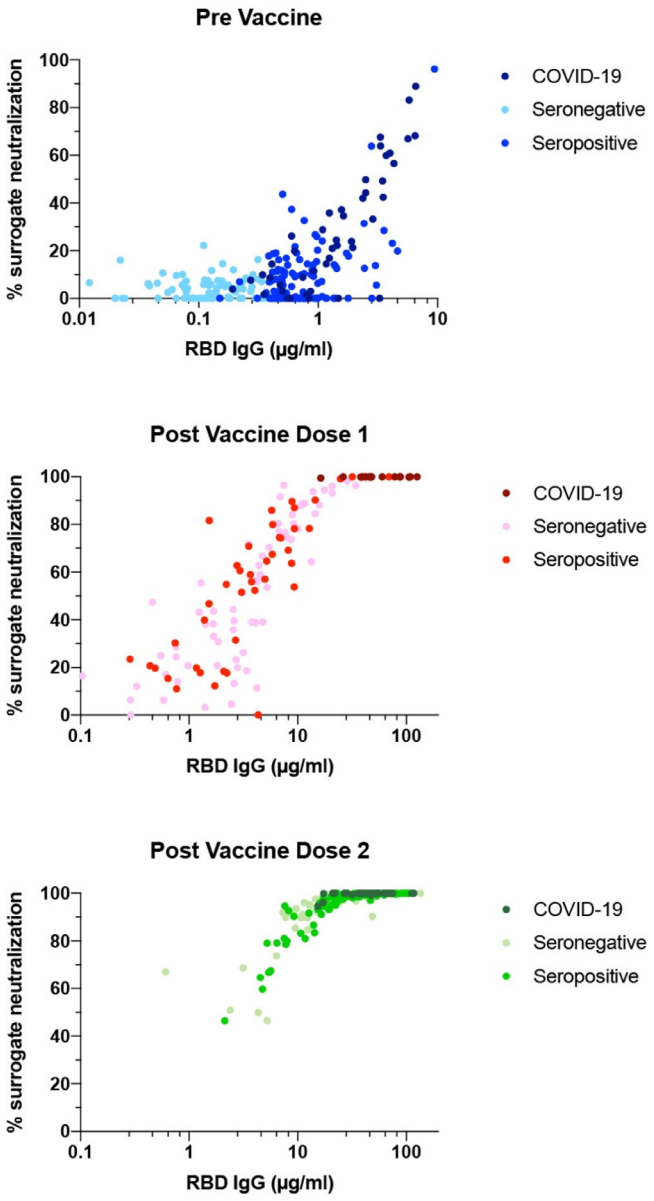

Fig. 2. Percentage surrogate neutralization positively correlates with anti-RBD IgG concentration. Samples were evaluated for both anti-RBD IgG levels and% surrogate neutralization of spike protein-ACE2 interaction before and after vaccination. Prior to vaccination anti-RBD IgG levels and% surrogate neutralization were positively correlated for COVID-19 (Spearman $r = 0{\cdot}79$, $p < 0{\cdot}001$) and seropositive participants (Spearman $r = 0{\cdot}23$, $p = 0{\cdot}012$). After one dose of COVID-19 vaccine, a wide range of IgG concentrations and% surrogate neutralization were observed in seropositive and seronegative samples, while all recovered COVID-19 samples reached > 95% neutralization (Spearman $r = 0{\cdot}59$, $p = 0{\cdot}020$ COVID-19; $r = 0{\cdot}85$, $p < 0{\cdot}001$ seropositive; $r = 0{\cdot}87$, $p < 0{\cdot}001$ seronegative). Anti-RBD IgG concentration and% surrogate neutralization increased dramatically after two doses of vaccine in the majority of seropositive and seronegative samples (Spearman $r = 0{\cdot}66$, $p < 0{\cdot}001$ COVID-19; $r = 0{\cdot}87$, $p < 0{\cdot}001$ seropositive; $r = 0{\cdot}89$, $p < 0{\cdot}001$ seronegative).

infection, suggest that seropositivity alone does not guarantee high levels of antibody-mediated protection following a single dose of the BNT162b2 and mRNA-1273 vaccines.

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Contributors

AD, LV, NR, MV, RH processed samples, performed ELISA assay. AS, TM processed samples and performed MSD Nab assay. AD, RS, DT, TM managed the data, analyzed data, and generated figures. TM, EM, BM, RS secured IRB approval and collected samples. AD, BM, TM, RD, EM, RS, DT provided critical input in study design and wrote the manuscript. All authors reviewed and approved the final version of the manuscript. All authors had full access to all the data in the study. AD, RD, BM, EM, TM had final responsibility for the decision to submit for publication.

Data sharing

Individual-level data of patients included in this manuscript after de-identification are considered sensitive. Requests for datasets should be made by qualified researchers trained in human subject confidentiality protocols to Dr. Thomas McDade at Northwestern University. The study method and statistical analyses are all described in detail in the Methods and throughout the manuscript.

Declaration of Competing Interest

Thomas McDade reports grants or contracts from the National Science Foundation, National Institutes of Health, and Office of Research (Northwestern University), and has a financial interest in EnMed Microanalytics, a company that specializes in laboratory testing of dried blood spot samples. Amelia Sancilio reports funding from the National Science Foundation. Brian Mustanski reports funding from the National Institutes of Health. Amelia Sancilio reports funding for her work via a grant received from the National Science Foundation, held by Thomas McDade. All other authors declare no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2021.101018.

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