

COVID-19 mRNA Vaccination Generates Greater Immunoglobulin G Levels in Women Compared to Men

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We investigated whether the antibody response to coronavirus disease 2019 (COVID-19) mRNA vaccination is similar in women and men. In a community cohort without prior COVID-19, first vaccine dose produced higher immunoglobulin G (IgG) levels and percent inhibition of spike-ACE2 receptor binding, a surrogate measure of virus neutralization, in women compared to men (7.0 µg/mL, 51.6% vs 3.3 µg/mL, 36.4%). After 2 doses, IgG levels remained significantly higher for women (30.4 µg/mL) compared to men (20.6 µg/mL), while percent inhibition was similar (98.4% vs 97.7%). Sex-specific antibody response to mRNA vaccination informs future efforts to understand vaccine protection and side effects.

Keywords. COVID-19; SARS-CoV-2; serological testing; IgG; ELISA; dried blood spots; vaccine; neutralizing; receptor binding domain.

Recently, the Centers for Disease Control and Prevention (CDC) reported women had more side effects than men after mRNA coronavirus disease 2019 (COVID-19) vaccination [1]. After COVID-19 infection, women mount a quicker and stronger immune response [2]. Antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike and nucleocapsid decline faster in men than women independent of age and body

mass index [3]. In addition, men present more frequently with severe COVID-19 and have a higher risk of death from COVID-19 compared to women, suggesting these antibody differences could contribute to differences in outcome [4, 5].

Two different SARS-CoV-2 spike mRNA vaccines, BNT162b2 and mRNA-1273, have been in use in the United States since December 2020 [6, 7]. Both vaccines have >94% efficacy in preventing symptomatic SARS-CoV-2 infections, but data are lacking on sex-specific responses to these mRNA vaccines. We compared antibody response to vaccination by sex among community participants without a prior clinical COVID-19 diagnosis. We quantified immunoglobulin G (IgG) to the spike receptor binding domain (RBD) and percent inhibition of spike-angiotensin-converting enzyme 2 (ACE2) receptor binding; the latter is a surrogate for virus neutralization activity. After 1 dose of mRNA vaccine, women had 2.1 times more anti-RBD IgG than men and had significantly increased inhibition of spike-ACE2 binding. After 2 doses of mRNA vaccine, women had 1.5 times more anti-RBD IgG antibodies compared to men. These results support a differential response to COVID-19 mRNA vaccine based on sex.

METHODS

Study Approval

The study was approved by the institutional review board at Northwestern University (No. STU00212457 and No. STU00212472).

Participants and Study Design

Nearly 8000 participants were recruited from the Chicago area from April to December 2020 [8]. Eligible participants provided informed consent electronically and completed an online survey regarding COVID-19 status. Materials for at-home self-collection of finger-stick dried blood spots (DBS) were shipped and returned through mail or on-site collection. A subset of participants (243 clinically reported SARS-CoV-2 positive, 1249 anti-RBD IgG seropositive, and 1534 randomly selected anti-RBD IgG seronegative) were recontacted regarding COVID-19 vaccination status from December 2020 until January 2021. Those that reported receiving 1 or 2 doses of an mRNA vaccine were similarly resampled. In total, 261 unique participants returned samples with 25 participants returning samples after both dose 1 and dose 2. We excluded those with history of a positive COVID-19 viral test for this analysis. DBS collected 0 to 9 days after dose 1 and 0 to 5 days after dose 2 were excluded.

Serological Assay

Anti-RBD IgG concentration was determined using an enzyme linked immunosorbent assay (ELISA) protocol on DBS

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eluates, as described [9, 10]. Assays were conducted blinded to participant health status. CR3022, an IgG antibody with a known affinity to the RBD of SARS-CoV-2 spike, was used as a multiconcentration standard curve to calculate the anti-RBD IgG concentration ($\mu\text{g}/\text{mL}$) in participant samples, using a 4-parameter logistic regression of the CR3022 calibration curve. A value $>0.39 \mu\text{g}/\text{mL}$ CR3022 was considered positive, as described [10]. This threshold is well above the assay lower limit of detection, is more than 3 standard deviations above values of known SARS-CoV-2 negative samples acquired before December 2019, and contained 28 of 30 clinically confirmed SARS-CoV-2 samples.

Spike-ACE2 Inhibition Assay

A matched aliquot was evaluated in the binding assay, as described [11]. Briefly, the assay was performed with slight modifications from the commercially available protocol (V-PLEX SARS-CoV-2 Panel 2 Kit; Meso Scale Diagnostics, K15386U-2). DBS samples were eluted overnight. Eluate ($25 \mu\text{L}$) was added to the assay plate well, which was precoated with SARS-CoV-2 spike protein (Wuhan variant). ACE2 protein ($25 \mu\text{L}$) conjugated to an electrochemiluminescent label was immediately added to the well and incubated for 1 hour. The plate was washed, read buffer added, and subsequently read using a MESO QuickPlex SQ 120MM Imager. The presence of inhibitory antibodies within the sample inhibits (neutralizes) spike protein binding to ACE2. Percent inhibition was calculated as follows: % inhibition = $100 \times 1 - (\text{sample signal}/\text{negative control signal})$. Samples were run in duplicate and reported as the average. Matched serum and DBS samples had high agreement (concordance correlation = 0.99) using this platform [11]. Previous work shows that surrogate virus neutralization test results correlate highly with results obtained from conventional live-virus neutralization tests (Pearson $R = 0.93$) and pseudovirus-based neutralization tests (Pearson $R = 0.92$) [12].

Statistical Analysis

Wilcoxon rank sum test with continuity correction was used to evaluate differences in antibody concentration and percent inhibition with $P < .05$ for significance. Spearman rank correlation was used to compare antibody concentration and percent inhibition. Normality was tested using the Komogorov-Smirnov test. The significance in differences between means for nonnormal distributions were estimated using a bootstrap approach where a random sample with replacement was taken and a sex stratified mean IgG value was computed.

RESULTS

Study groups were created based on reported sex assigned at birth (Table 1). Both groups, women and men, had similar percentages of prevaccination anti-RBD IgG seropositive and seronegative samples without clinically diagnosed COVID-19: after dose 1, 36.5% of women and 35.7% of men were seropositive

and after dose 2, 49.0% of women and 52.6% of men were seropositive (Table 1). Prior to vaccination, the median anti-RBD IgG concentration and percent inhibition were similar between the women ($0.3 \mu\text{g}/\text{mL}$, 2%) and men ($0.2 \mu\text{g}/\text{mL}$, 4%) groups ($P = .54$ and $P = .25$, respectively). For dose 1, both the women and men had an equal percentage of samples from each manufacturer (women 56.5% BNT162b2, 42.4% mRNA-1273; men 59.5% BNT162b2, 40.5% mRNA-1273) (Table 1). Following the first dose of mRNA vaccine (BNT162b2 $n = 73$; mRNA-1273 $n = 53$), there was a significant increase in anti-RBD IgG and percent inhibition in both groups. The mean IgG concentration was 2-fold higher in women compared to men ($7.0 \mu\text{g}/\text{mL}$ women, $3.3 \mu\text{g}/\text{mL}$ men; $P < .01$; Figure 1A). The median anti-RBD IgG concentration following the first dose of mRNA vaccine in the women compared to men did not reach statistical significance ($2.9 \mu\text{g}/\text{mL}$ women, $2.7 \mu\text{g}/\text{mL}$ men; $P = .13$). The difference in statistical significance of the women's mean and median values can be explained by the longer right tail of the women group distribution (skewness 3.18 women, 0.84 men; confidence interval 1.1–4.4; $P < .01$). Percent inhibition following the first mRNA vaccine dose was significantly higher for women compared to men (51.6% women, 36.4% men; $P = .02$; Figure 1A). Anti-RBD levels positively correlated with percent inhibition in this assay with 8 of 85 samples from women reaching greater than 95% inhibition compared to 0 of 42 samples from men (Figure 1B).

Following administration of the second mRNA vaccine dose (women 93.1% BNT162b2, 6.9% mRNA-1273; men 93.0% BNT162b2, 7.0% mRNA-1273), there were significant increases in anti-RBD IgG concentration and percent inhibition for both groups (Table 1). In women after the second mRNA dose, median IgG concentration increased compared to dose 1 by a factor of 10.5 ($P < .001$), while for men the median IgG concentration increased compared to dose 1 by a factor of 13.5 ($P < .001$). Median anti-RBD IgG concentration significantly differed by sex following the second mRNA vaccine dose ($30.4 \mu\text{g}/\text{mL}$ women, $20.6 \mu\text{g}/\text{mL}$ men; $P = .02$), equivalent to 1.5-fold more anti-RBD IgG in women compared to men (Figure 1C). Median anti-RBD IgG concentration after 2 doses of mRNA vaccine was similar between seronegative and seropositive persons without a history of clinical COVID-19 diagnosis within both the groups of women and men (women, median anti-RBD IgG $39.12 \mu\text{g}/\text{mL}$ seronegative, $37.35 \mu\text{g}/\text{mL}$ seropositive, $P = .84$; men, median anti-RBD IgG $18.92 \mu\text{g}/\text{mL}$ seronegative, $22.46 \mu\text{g}/\text{mL}$ seropositive $P = .60$). Percent inhibition following the second mRNA vaccine dose was comparable between women and men (98.4 % women, 97.7 % men; $P = .36$; Figure 1C). Anti-RBD levels positively correlated with percent inhibition with 72 of 102 (70%) women reaching greater than 95% inhibition compared to 40 of 57 (70%) men when sampled 6 or more days after the second vaccine dose (Figure 1D).

Table 1. Sample Characteristics by Assigned Birth Sex

Characteristic	Women			Men		
	Prevaccine (n = 175)	Dose 1 (n = 85)	Dose 2 (n = 102)	Prevaccine (n = 86)	Dose 1 (n = 42)	Dose 2 (n = 57)
Age, median (IQR)	37.0 (30.0–48.0)	40.0 (30.0–59.0)	34.5 (28.0–43.0)	42.0 (31.0–58.0)	43.5 (34.0–64.0)	39.0 (30.0–49.0)
Serostatus, n (%)						
Seropositive	77 (44.0)	31 (36.5)	50 (49.0)	40 (53.5)	15 (35.7)	30 (52.6)
Seronegative	98 (56.0)	54 (63.5)	52 (51.0)	46 (46.5)	27 (64.3)	27 (47.4)
Race, n (%)						
Hispanic/Latinx	33 (18.9)	13 (15.3)	21 (20.6)	8 (9.3)	4 (9.5)	4 (7.0)
Non-Hispanic Asian	30 (17.1)	12 (14.1)	18 (17.7)	12 (14.0)	5 (11.9)	10 (17.5)
Non-Hispanic black	7 (4.0)	5 (5.9)	2 (2.0)	1 (1.2)	0 (0.0)	1 (1.8)
Non-Hispanic white	98 (56.0)	51 (60.0)	58 (56.9)	62 (72.1)	32 (76.2)	39 (68.4)
Non-Hispanic other	7 (4.0)	4 (4.7)	3 (2.9)	3 (3.5)	1 (2.4)	3 (5.3)
Vaccine/manufacture, n (%)						
BNT162b2/Pfizer	134 (76.6)	48 (56.5)	95 (93.1)	69 (80.2)	25 (59.5)	53 (93.0)
mRNA-1273/Moderna	40 (22.9)	36 (42.4)	7 (6.9)	17 (20.0)	17 (40.5)	4 (7.0)
Missing	1 (0.6)	1 (1.2)	0 (0.0)	0	0	0
Immunoglobulin G, µg/mL, median (IQR)	0.3 (0.1–0.6)	2.9 (0.8–8.8)	30.4 (16.0–55.5)	0.2 (0.0–0.7)	2.7 (0.3–5.0)	20.6 (13.0–33.4)
Neutralizing antibody percent, median (IQR)	2.0 (0.0–8.2)	51.6 (20.7–78.3)	98.4 (93.1–99.8)	4.0 (0.0–11.0)	36.4 (11.4–64.6)	97.7 (94.0–99.4)
DBS sample acquisition						
Days between prevaccine and first dose, median (IQR)	...	92.0 (81.0–152.0)	117.5 (86.5–167.5)	...	120.5 (74.0–176.0)	149.0 (120.0–183.0)
Days since receipt of vaccine dose, median (IQR)	...	18.0 (14.0–21.0)	40.0 (32.0–48.5)	...	18.0 (12.0–20.0)	45.0 (39.0–52.0)

Abbreviations: DBS, dried blood spot; IQR, interquartile range.

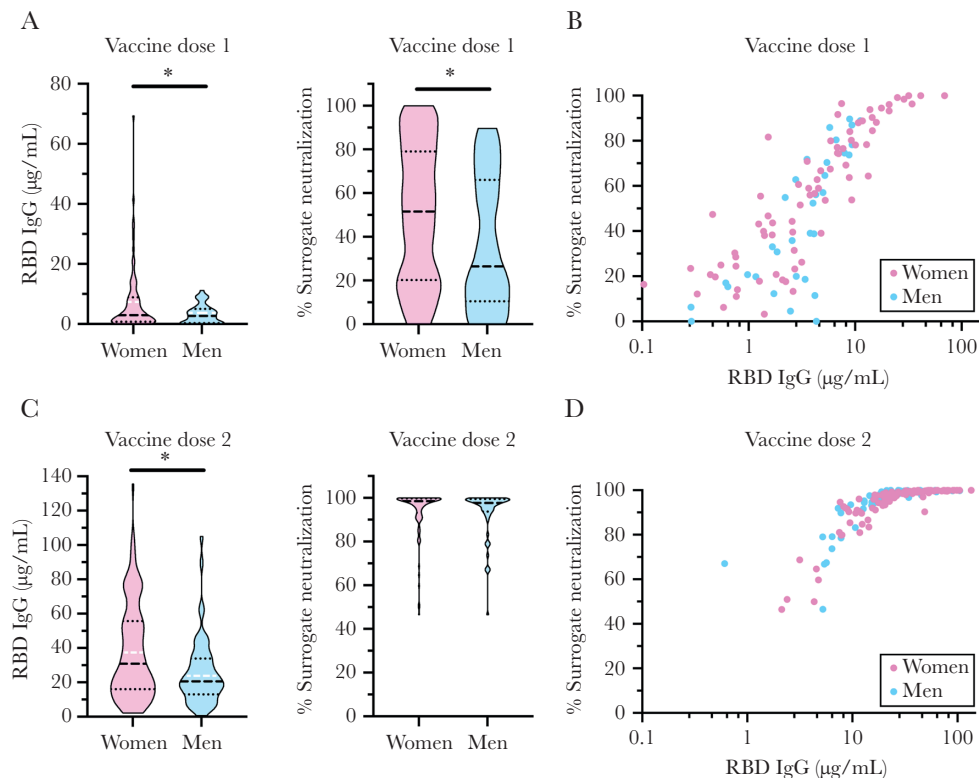


Figure 1. Antibody responses after 1 or 2 doses of mRNA vaccine by sex. *A*, Dried blood spots were evaluated after the first dose of a COVID-19 mRNA vaccine (either BNT162b2, $n = 73$, or mRNA-1273, $n = 53$). The mean IgG concentration was 2-fold higher in women compared to men (7.0 $\mu\text{g/mL}$ women, 3.3 $\mu\text{g/mL}$ men; $*P < .01$ [white dashed lines]), while the median did not reach significance in women compared to men (2.9 $\mu\text{g/mL}$ women, 2.7 $\mu\text{g/mL}$ men; $P = .13$ [black dashed lines]). Percent inhibition of spike-ACE2 interaction was significantly higher in women compared to men (51.6% women, 36.4% men; $*P = .02$). Black dotted lines denote 25th and 75th percentiles. *B*, Anti-RBD levels positively correlated with percent inhibition (Spearman $r = 0.90$ women, $n = 85$; $r = 0.80$ men, $n = 42$). *C*, Dried blood spots were evaluated after 2 doses of a COVID-19 mRNA vaccine (either BNT162b2, $n = 148$, or mRNA-1273, $n = 11$). Both the mean and median anti-RBD IgG concentrations were significantly increased in women compared to men (mean 37.5 $\mu\text{g/mL}$ women, 25.7 $\mu\text{g/mL}$ men; $*P = .002$ [white dashed lines]; median 30.4 $\mu\text{g/mL}$ women, 20.6 $\mu\text{g/mL}$ men; $*P = .02$ [black dashed lines]). Percent inhibition of spike-ACE2 interaction was not different between women and men (98.4% women, 97.7% men; $P = .36$) with 70% of samples over 95% inhibition in both groups. Black dotted lines denote 25th and 75th percentiles. *D*, Anti-RBD levels positively correlated with percentage surrogate neutralization (Spearman $r = 0.92$ women, $n = 102$; $r = 0.84$ men, $n = 57$). Abbreviations: ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; IgG, immunoglobulin G; RBD, receptor-binding domain.

DISCUSSION

Vaccination to protect against COVID-19 is rapidly escalating in the United States and around the world to reduce COVID-19 hospitalizations and death, among many other benefits. To date, more than 160 million doses of mRNA vaccines have been administered in the United States under an emergency use authorization granted by the Food and Drug Administration. During the first month of vaccine reporting data, the V-safe program conducted by the CDC noted the majority of adverse effects after vaccination were reported by women (79.1%) when approximately 60% of vaccine recipients were women [1]. Although many factors can account for this difference, similar sex-based responses to vaccines have been noted with other vaccine types, and this has been attributed to greater immune responses generally in women compared to men [13, 14].

In community participants with no prior history of COVID-19, women had higher levels of mean anti-RBD IgG after vaccination compared to men. This difference was

apparent after the first dose of vaccine and it persisted after the second dose. Percent inhibition of spike-cellular ACE2 receptor interaction was also significantly higher in women after the first mRNA vaccine dose. Both groups reached a high percent inhibition after the second dose, which could reflect maximal limits of the assay. However, results here and elsewhere show consistent strong associations between key anti-SARS-CoV-2 spike epitope-directed IgG levels, including the anti-RBD IgG assessed here, and neutralization of virus entry in cell culture [12, 15]. These data suggest additional analysis of studies by sex are warranted, to better understand persistence of the vaccine response and guide strategies for future booster vaccination.

Notes

Disclaimer. The funding sources had no role in the study design, data collection, analysis, interpretation, or writing of the report.

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Potential conflicts of interest. T. M. has a financial interest in EnMed Microanalytics, a company that specializes in laboratory testing of dried blood spot samples. All other authors declare no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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