Supplemental Online Content

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This supplemental material has been provided by the authors to give readers additional information about their work.

eMethods. MATERIALS AND METHODS

Study design and participants

This study was designed to test the blood antibody levels to the SARS-CoV-2 Spike (S-protein) and nucleocapsid (N-protein) proteins in patients receiving the Pfizer/BioNTech (BNT162b2) vaccine. Three (3) time points (baseline, 3 weeks after the first vaccine dose, and 4 weeks post second vaccine dose) were collected for each participant. Study participants were enrolled at Rush University Medical Center (IRB: 20081004-IRB01 and 21010601-IRB01) and provided informed consent to provide up to 20 ml of venous blood from which plasma was isolated, deidentified, and shipped to Abbott Diagnostics (Abbott Park, IL) on dry ice for testing. Patients also provided demographic data including age (years), race/ethnicity (B=Black, W=White, A=Asian, H=Hispanic), sex (Male/Female), and history of COVID-19 infection including symptoms and SARS-CoV-2 test results (Supplemental Table 1). Participants were excluded if they reported being immunocompromised, had received SARS-CoV-2 monoclonal antibody therapy, or had received SARS-CoV-2 convalescent plasma. In total 59 participants were recruited for the study. Baseline screening testing and patient history questionnaires identified 30 participants with no evidence of prior SARS-CoV-2 infection. One participant in this cohort was unable to return for their second dose follow up sample. Baseline screening testing and patient history questionnaires identified 29 patients with evidence of previous SARS-CoV-2 infection. Within this cohort, 2 participants were unable to provide baseline samples, 2 participants did not provide a sample for the 3 weeks post first vaccine dose, and 3 participants were unable to return for the 4 weeks post second dose follow up visit. All study participants provided samples for at least 2 of the 3 timepoints to be included for analysis.

SARS-CoV-2 antibody detection

All samples were run on an Abbott ARCHITECT™ *i*2000SR instrument and tested with a panel of three Abbott Laboratories SARS-CoV-2 antibody assays as described below. The FDA EUA approved SARS-CoV-

2 IgG (List 6R86), AdviseDx SARS-CoV-2 IgM (List 6R87), and the CE marked SARS-CoV-2 IgG II Quant (List 6S60) assays were performed according to the ARCHITECT operations manual and assay package insert instructions. Briefly, the SARS-CoV-2 IgG and IgM assays are automated Chemiluminescent Microparticle Immunoassays (CMIA) used for the qualitative detection of IgG antibodies directed against the SARS-CoV-2 N-protein and IgM antibodies against the S-protein respectively. Assay results are measured in Relative Light Units (RLU) and reported as an index value of the ratio of specimen to calibrator RLU signal (S/C or S/Co). Index values ≥1.4 S/C indicate a SARS-CoV-2 IgG seropositive result and index values ≥1.0 S/C indicate a SARS-CoV-2 IgM seropositive result. The diagnostic utility of the ARCHITECT SARS-CoV-2 IgG [5-7] and ARCHITECT SARS-CoV-2 IgM [8] assays have been previously reported. The SARS-CoV-2 IgG II Quant assay is an automated CMIA used for the quantitative detection of IgG antibodies directed against the receptor binding domain (RBD) of the SARS-CoV-2 S-protein with assay results reported in AU/mL. Assay calibration is performed using 6-point calibration referencing an internal reference standard at each concentration level. Assay linearity was shown between 21.0 and 40,000 AU/mL, with results <50.0 AU/mL reported as negative and ≥50.0 reported as positive. Results ≥40,000 AU/mL were diluted, retested, and corrected for dilution factor.

Statistical Analysis

Study participants (n=59) were grouped into two different cohorts based on previous evidence of SARS-CoV-2 infection (previous positive PCR, antibodies against S- and/or N-proteins at baseline). In total, 30 participants did not have evidence of previous SARS-CoV-2 infection and 29 participants did. To be included for analysis, participants needed to have samples collected at 2 of the 3 timepoints. All graphing and statistical analysis was conducted with GraphPad Prism Version 8.0.2. Specifically, an unpaired two-tailed *t* test was used to assess differences in SARS-CoV-2 IgG levels between vaccine timepoints in the cohorts described above. Significance was defined as $p<0.05$ and the exact values out to 4 decimal places is reported. Descriptive statistics such as minimum, maximum, mean, median, and

standard deviation was also computed in GraphPad Prism. Age vs antibody response graphs including best-fit lines, 95% confidence intervals, R square, and slope *p* values were calculated using Prism's linear regression analysis. A secondary statistical analysis was conducted which restricted the analysis to the subset of participants for which all 3 timepoints were collected (n=29 previously uninfected and n=22 with evidence of prior infection). One-way ANOVA with Tukey's multiple comparisons analysis of previously uninfected individuals resulted in statistical significance between baseline and 1 vaccine dose (p<0.0001), baseline and 2 vaccine doses (p<0.0001), and 1 dose vs 2 doses (p<0.0001). One-way ANOVA with Tukey's multiple analysis of individuals with evidence of previous SARS-CoV-2 infection resulted in statistical significance between baseline and 1 vaccine dose (p=0.0078), baseline and 2 vaccine doses (p=0.0036), and 1 dose vs 2 doses (p=NS). Restricting the analysis to only participants with all 3 timepoints did not change the interpretation of the data, so all participants with at least 2 of 3 timepoints are reported.

eTable. Participant Demographics

Gender	Age (years)	Reported race or ethnicity	Date of COVID-19 symptom onset or positive SARS-CoV-2 PCR	Days from symptom onset or +PCR to first dose of vaccine
Subjects with evidence of previous SARS-CoV-2 infection (n=29)				
F	46	B	3/13/2020	306
F	50	A	4/25/2020	247
F	47	W	3/11/2020	293
F	27	A	10/16/2020	67
F	41	A	5/14/2020	220
F	41	W	4/15/2020	246
F	27	H	9/21/2020	88
F	38	B	N/A	N/A
F	27	W	11/19/2020	28
F	36	H	10/29/2020	67
F	48	W	11/6/2020	55
M	59	W	3/20/2020	276
M	47	H	9/19/2020	101
F	36	W	7/9/2020	172
F	57	W	3/4/2020	301

M	63	W	5/17/2020	232
F	29	W	11/10/2020	55
M	22	B	N/A	N/A
F	44	W	3/7/2020	298
F	43	H	11/25/2020	36
F	24	W	11/18/2020	43
F	42	A	5/6/2020	226
M	42	W	11/6/2020	68
F	44	A	12/12/2020	26
M	35	H	11/10/2020	50
M	64	W	11/9/2020	59
M	57	W	N/A	N/A
F	25	A	N/A	N/A
F	34	W	10/16/2020	75
Subjects without evidence of previous SARS-CoV-2 infection (n=30)				
M	61	W	N/A	N/A
F	29	W	N/A	N/A
M	53	W	N/A	N/A
F	63	W	N/A	N/A
M	42	A	N/A	N/A
F	59	A	N/A	N/A
F	46	B	N/A	N/A
F	41	B	N/A	N/A
F	39	A	N/A	N/A
M	38	W	N/A	N/A
F	43	H	N/A	N/A
F	34	A	N/A	N/A
F	33	A	N/A	N/A
F	30	A	N/A	N/A
F	37	W	N/A	N/A
F	39	A	N/A	N/A
M	65	A	N/A	N/A
F	30	W	N/A	N/A
M	46	W	N/A	N/A
F	28	W	N/A	N/A
F	38	W	N/A	N/A
F	35	B	N/A	N/A
F	30	W	N/A	N/A
F	32	B/W	N/A	N/A
F	57	A	N/A	N/A
F	44	W	N/A	N/A
F	32	H	N/A	N/A
M	65	W	N/A	N/A

F	49	B	N/A	N/A
M	67	W	N/A	N/A