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Authors

Tang, Li
Cherry, Sean
Tuomanen, Elaine
[et al.](#)

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Host Predictors of Broadly Cross-Reactive Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Variants of Concern Differ Between Infection and Vaccination

Li Tang,^{1,a} Sean Cherry,^{2,a} Elaine I. Tuomanen,² Ericka Kirkpatrick Roubidoux,² Chun-Yang Lin,³ Kim J. Allison,² Ashleigh Gowen,² Pamela Freiden,² E. Kaitlynn Allen,³ Yin Su,¹ Aditya H. Gaur,² Jeremie H. Estep,^{4,5} Maureen A. McGargill,^{3,6} Florian Kramer,⁶ Paul G. Thomas,³ Stacey Schultz-Cherry,^{2,6} and Joshua Wolf^{2,6}; for the St. Jude Investigative Team^b

¹Department of Biostatistics, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; ²Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; ³Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; ⁴Department of Hematology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; ⁵Department of Global Pediatric Medicine, St. Jude Children's Research Hospital, Memphis, Tennessee, USA; and ⁶Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York, USA

Background. Following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection or vaccination there is significant variability between individuals in protective antibody levels against SARS-CoV-2, and within individuals against different virus variants. However, host demographic or clinical characteristics that predict variability in cross-reactive antibody levels are not well-described. These data could inform clinicians, researchers, and policymakers on the populations most likely to require vaccine booster shots.

Methods. In an institutional review board–approved prospective observational cohort study of staff at St. Jude Children's Research Hospital, we identified participants with plasma samples collected after SARS-CoV-2 infection, after mRNA vaccination, and after vaccination following infection, and quantitated immunoglobulin G (IgG) levels by enzyme-linked immunosorbent assay to the spike receptor binding domain (RBD) from 5 important SARS-CoV-2 variants (Wuhan Hu-1, B.1.1.7, B.1.351, P.1, and B.1.617.2). We used regression models to identify factors that contributed to cross-reactive IgG against 1 or multiple viral variants.

Results. Following infection, a minority of the cohort generated cross-reactive antibodies, IgG antibodies that bound all tested variants. Those who did had increased disease severity, poor metabolic health, and were of a particular ancestry. Vaccination increased the levels of cross-reactive IgG levels in all populations, including immunocompromised, elderly, and persons with poor metabolic health. Younger people with a healthy weight mounted the highest responses.

Conclusions. Our findings provide important new information on individual antibody responses to infection/vaccination that could inform clinicians on populations that may require follow-on immunization.

Keywords. antibody response; BMI; metabolic health; SARS-CoV-2; variants of concern.

Since its emergence in December 2019, there have been more than 198 million cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection with over 4.2 million deaths (World Health Organization [1]). Viral evolution has caused considerable genetic change compared with the original SARS-CoV-2 Wuhan Hu-1 strain [2]. These genetic changes include substitutions within the functional domains of the viral spike (S) protein leading to altered biological properties,

including increased transmissibility, enhanced binding to the human angiotensin-converting enzyme 2 (ACE2) receptor, and increasing virulence [2]. Important variants of concern (VOCs) include B.1.1.7 (Alpha), detected in the United Kingdom in September 2020; B.1.351 (Beta), detected in South Africa in May 2020; P.1 (Gamma), detected in Brazil in November 2020; and B.1.617.2 (Delta), detected in India in October 2020 [3]. As the virus continues to mutate, a better understanding of the breadth of the antibody response following infection or vaccination is critical for future vaccine design and public health interventions.

Studies have focused on assessing antibody responses to a specific VOC or set of samples—for example, convalescent patients or vaccine recipients [4–6]. These studies found reduced binding and neutralization against variants, further exemplified by a comprehensive study [7] examining antibody potency against all VOCs using convalescent samples from patients with

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^aL. T. and S. C. contributed equally to this manuscript.

^bSt. Jude Investigative Team members are listed in the Acknowledgments.

Correspondence: J. Wolf, Associate Member, Department of Infectious Diseases, St. Jude Children's Research Hospital, MS320, 262 Danny Thomas Place, Memphis, TN 38105, USA (joshua.wolf@stjude.org).

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varying disease severity, and mRNA vaccine recipients, and another showing that vaccination increases all components of the humoral response [8]. However, individual immunoglobulin G (IgG) responses differ, and recent studies showed that serum samples from convalescent patients display variable neutralization capacities [9]. It remains unclear whether specific host characteristics predict antibody breadth and magnitude against the VOCs.

To fill this knowledge gap, we used enzyme-linked immunosorbent assay (ELISA) to quantitate IgG titers against the spike receptor binding domain (RBD) from SARS-CoV-2 Wuhan Hu-1, B.1.1.7, B.1.351, B.1.617.2, and P.1 strains in plasma samples collected following infection and/or vaccination from a cohort of 399 healthcare workers enrolled in a prospective study.

METHODS

Data Availability

Authors confirm all relevant data are included in the paper and/or supplementary files.

Study Participants and Measures

The St. Jude Tracking of Viral and Host Characteristics Associated with COVID-19 Study (SJTRC; NCT04362995) is a prospective, institutional review board–approved, longitudinal cohort study of adult (≥ 18 years of age) employees at St. Jude Children’s Research Hospital initiated in March 2020. Participants provided written informed consent, and completed questionnaires about demographics, medical history, treatment, and symptoms, and a severity questionnaire following polymerase chain reaction (PCR)–confirmed SARS-CoV-2 infection. Participants were screened for SARS-CoV-2 by nasal swab PCR weekly when on campus, and convalescent blood was collected approximately 3–8 weeks after SARS-CoV-2 infection and/or vaccination. Blood samples were separated into cellular and plasma components within 24 hours of collection and stored at -80°C . Severity of disease was dichotomized as follows: severe or critical (dyspnea, or requiring supplemental oxygen, hospital admission, or dialysis during the COVID-19 episode) versus others. Body mass index (BMI) was self-reported, classified according to Centers for Disease Control and Prevention criteria, and dichotomized as obese or very obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) versus others [10]. Immunocompromise included all immunocompromising conditions as reported by participants, including autoimmune diseases, antibody deficiency, and corticosteroid use (Supplementary Table 1). Participants who had completed a full course of BNT162b2 by the data freeze were included in postvaccine cohorts.

Receptor Binding Domain (RBD) ELISA

Purified SARS-CoV-2 Wuhan Hu-1, B.1.1.7, B.1.351, P.1, and B.1.617.2 RBD proteins, expressed in 293T cells as described [11, 12], were diluted to a concentration of $1.5 \mu\text{g/mL}$ in

phosphate-buffered saline (PBS) and added at $50 \mu\text{L}$ per well to a 96-well ELISA plate (Immulon 4 HBX; ThermoFisher Scientific). The ELISA plates were sealed and allowed to incubate at 4°C overnight, as described [12]. The next day the coating solution was removed, and the plates were blocked at room temperature using 3% nonfat milk prepared in PBS ($200 \mu\text{L}/\text{well}$) for 1–4 hours. While the plates were being blocked, the samples were prepared by diluting the heat-inactivated plasma 1:50 in 1% fat dry milk. Following blocking, the milk was removed, and the plates were washed 3 times with PBS containing 0.1% Tween-20 (PBST) at $200 \mu\text{L}$ per well. The diluted plasma was added to the blocked plate at $50 \mu\text{L}$ per well and incubated for 1.5 hours at room temperature and then removed and washed 3 times with $200 \mu\text{L}$ 0.1% PBST. Goat anti-human IgG horseradish peroxidase (HRP) conjugated secondary antibody (ThermoFisher Scientific) was diluted 1:2500 in 1% fat dry milk and $50 \mu\text{L}$ was added to each well of the washed plate and incubated at room temperature for 30 minutes. Following the incubation period, the secondary antibody was removed, and the plate was washed 3 times with 0.1% PBST. SIGMAFAST OPD (o-phenylenediamine dihydrochloride; Sigma-Aldrich) substrate was prepared directly before use and added at $50 \mu\text{L}$ per well for exactly 8 minutes. The OPD substrate was stopped by adding $50 \mu\text{L}$ of 3M hydrochloric acid. The optical density at 490 nm (OD490) was measured using a Synergy 4 (BioTek) plate reader. SARS-CoV-2 reactive human monoclonal antibody CR3022 [11] at 1:5000, 1:25 000, 1:125 000, and 1:625 000 dilutions and plasma from naturally infected donors at a 1:50 dilution and known negative, prepandemic plasma samples diluted at 1:50 were used as controls. The background value was set at an OD490 of 0.15, as previously described [11]. Samples with an OD490 2 times greater than background, or 0.3 or greater, were considered positive as this value has been suggested as a surrogate for the presence of neutralizing antibodies in the same assay [12].

Adiponectin and Leptin ELISA

Adiponectin and leptin concentrations were measured in heat-inactivated plasma diluted at 1:50 or 1:5000 using the R&D Systems Leptin and Adiponectin DuoSet ELISA, respectively, per the manufacturer’s protocol. An adiponectin to leptin ratio greater than 1.0 was defined as at low risk for adipose tissue dysfunction or metabolic syndrome, more than 0.5 to less than 1.0 as moderate risk, and less than 0.5 as high risk [11, 13–15]. For the statistical analyses, the ratio was dichotomized as high risk versus others.

Statistical Analysis

This study comprised a convenience subcohort of 399 participants from the larger study, with 581 relevant samples selected to represent important antigen-exposure paradigms. Although not prospectively powered for this objective, this analysis

had greater than 80% power to detect an OD fold-change of 1.3 or greater or 0.77 or less, using a *t* test with a coefficient of variation of 1 and a ratio of 2:1 vaccination versus infection only [16]. Initial descriptive analysis estimated medians and interquartile ranges (IQRs) of OD values in each group, proportions with error bars for categorical variables, and means with standard deviations for continuous variables. Chi-square test or a univariate logistical model was implemented to compare categorical variables if the count was not sparse; otherwise, Fisher's exact test was used. Wilcoxon signed-rank test was performed to compare continuous measures. After descriptive analysis, OD values were natural log-transformed. Ward's hierarchical agglomerative clustering method [17] using Spearman's correlation as the distance measure for transformed OD values and using Euclidean distance for covariates was applied to examine the pattern of OD values. Infection or vaccination was a split variable in clustering. Multivariate linear effects models on natural log-transformed OD values were used to investigate associations of host characteristics with antibody levels. Host characteristics, including gender, age, race, ethnicity, immunocompromise, BMI classification, adiponectin to leptin (Adp:Lep) ratio, patient contact, time since infection diagnosis or vaccination completion, infection severity, and time of infection (before or after 1 October 2020), were considered as covariate candidates. The date cutoff of 1 October 2020 was selected arbitrarily as being between the first and second epidemic waves in Memphis, Tennessee. The elastic net model selection method [18], which extends the LASSO method [19] and ridge regression [20], was carried out with the Bayesian information criterion (BIC) [21] applied. For analysis of cross-reactivity only, samples with an OD value of 0.3 or greater were considered positive as this value has been suggested as a surrogate for the presence of neutralizing antibodies in the same assay [22]. Cross-reactivity was defined as the presence of antibodies (OD \geq 0.3) against Wuhan Hu-1 or B.1.1.7, plus either B.1.351 or P.1 variants. Multivariate logistic models were implemented to analyze cross-reactivity, with a similar model selection strategy. Multivariate linear or logistic mixed models were also attempted, but were not included because estimates were very close, and the BIC consistently favored the former. A 2-sided *P* value less than .05 was considered significant. Statistical analyses were performed in SAS software, version 9.4 (SAS Institute, Inc.), and R software, version 4.0.2 (R Foundation for Statistical Computing).

RESULTS

Cohort Selection

Participants were enrolled into SJTRC, a prospective longitudinal cohort study of adult (\geq 18 years of age) employees at St. Jude Children's Research Hospital between March 2020 and March 2021. Participants were screened for SARS-CoV-2 by

nasal swab PCR approximately weekly or if symptomatic and, of 1315 participants, 191 had documented SARS-CoV-2 infection prior to June 2021. (Supplementary Table 1). The included subcohort of 399 participants included in this analysis comprised 120 with infection alone, 237 with vaccination alone, and 42 who had vaccination after infection. Subcohort characteristics are shown in Table 1 and Supplementary Tables 1 and 2 and were similar to the overall cohort.

Most participants with SARS-CoV-2 infection had mild or moderate illness with viruses derived from Wuhan-like viruses or B.1 derivative lineages that did not contain known markers of substantial antigenic effect (Supplementary Table 9). Severe or critical coronavirus disease 2019 (COVID-19) was associated with immunocompromise (odds ratio [OR] = 6.9; *P* = .016) and obesity (BMI \geq 30 kg/m², OR = 2.64; *P* = .0085). Lower Adp:Lep ratio also correlated with disease severity (Table 2); 70% of participants with critical or severe disease had Adp:Lep less than 0.5, signifying adipose tissue dysfunction. There was no association with gender, race, age, ethnicity, or patient contact (Table 2 and Supplementary Table 3).

Characteristics Associated With Generation of Cross-protective IgG During Infection

To define factors underlying the magnitude and breadth of the IgG response against Wuhan Hu-1, B.1.1.7, B.1.351, B.1.617.2, and P.1 RBDs produced in response to infection, vaccination, or vaccination postinfection, 581 evaluable samples were tested for IgG against the RBD of SARS-CoV-2 variants by ELISA. ODs less than 0.3 were considered negative for the cross-reactivity analysis. Median IgG titers against SARS-CoV-2 variants were highest in samples collected after completion of SARS-CoV-2 vaccination, with titers against Wuhan Hu-1 and B.1.617.2 higher than the other 3 VOCs (Figure 1).

Host Characteristics Associated With Broadly Reactive IgG Responses After Infection Include Poor Metabolic Health

Hierarchical clustering was used to identify factors underlying the cross-reactive antibodies. We classified individual VOC RBD IgG titers to infection (Figure 2) and vaccination (Figure 3) and examined host characteristics associated with high-magnitude, cross-reactive IgG responses. Following SARS-CoV-2 infection, time since infection (>14 days vs \leq 14 days), more severe illness, and receipt of any SARS-CoV-2 vaccine were positively associated with higher IgG against all SARS-CoV-2 RBDs (Figure 2, Supplementary Table 4, and Supplementary Figure 1) or, in the case of disease severity, 3 of 4 VOCs: B.1.351, B.1.617.2, and P.1 (Figure 4 and Supplementary Table 5).

A low Adp:Lep, indicative of poor metabolic health, was also associated with higher cross-reactive IgG titers (Figure 2, Supplementary Table 4, and Supplementary Figure 1). We hypothesized that this was related to the increased disease severity observed in this group (Table 2). However, the effect of adipose

Table 1. Characteristics of Included Participants

	SARS-CoV-2 Infection Only (n = 120)		SARS-CoV-2 Vaccination Only (n = 237)		Vaccination After SARS-CoV-2 Infection (n = 42)	
	n	(%)	n	(%)	n	(%)
Gender						
Female	94	(78.3%)	186	(78.5%)	34	(81%)
Male	26	(21.7%)	51	(21.5%)	8	(19%)
Age, mean (SD), years	41.2 (11.2)		47 (12.3)		45.8 (12.2)	
Race (self-reported)						
White/Caucasian	89	(74.2%)	214	(90.3%)	36	(85.7%)
Black/African American	18	(15%)	13	(5.5%)	4	(9.5%)
Asian	8	(6.7%)	6	(2.5%)	1	(2.4%)
Other	4	(3.3%)	3	(1.3%)	0	(0%)
Not reported	1	(0.8%)	1	(0.4%)	1	(2.4%)
Hispanic ethnicity	5	(4.2%)	6	(2.5%)	1	(2.4%)
Immunocompromise	6	(5%)	9	(3.8%)	1	(2.4%)
Patient contact						
None	66	(55%)	62	(26.2%)	13	(31%)
Indirect	12	(10%)	42	(17.7%)	7	(16.7%)
Direct	42	(35%)	133	(56.1%)	22	(52.4%)
BMI classification						
Underweight (<18.5 kg/m ²)	1	(0.8%)	5	(2.1%)	1	(2.4%)
Normal weight (18.5–<25.0 kg/m ²)	34	(28.3%)	97	(40.9%)	13	(31%)
Overweight (25.0 to <30 kg/m ²)	30	(25%)	72	(30.4%)	12	(28.6%)
Obese (30 to <40 kg/m ²)	35	(29.2%)	50	(21.1%)	14	(33.3%)
Very obese (≥40 kg/m ²)	11	(9.2%)	8	(3.4%)	2	(4.8%)
Not reported	9	(7.5%)	5	(2.1%)	0	(0%)
Adiponectin to leptin ratio						
Normal (≥1)	32	(26.7%)	88	(37.1%)	11	(26.2%)
Abnormal (0.5 to <1)	15	(12.5%)	35	(14.8%)	10	(23.8%)
Very abnormal (<0.5)	73	(60.8%)	113	(47.7%)	21	(50%)
Not available	0	(0%)	1	(0.4%)	0	(0%)
Infection severity						
Asymptomatic	10	(8.3%)		N/A	2	(4.8%)
Mild–moderate	67	(55.8%)		N/A	33	(78.6%)
Severe	36	(30%)		N/A	7	(16.7%)
Critical	2	(1.7%)		N/A	0	(0%)
Not reported	5	(4.2%)		N/A	0	(0%)

Abbreviations: BMI, body mass index; N/A, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation.

tissue dysfunction on IgG levels was not mediated by severity of infection, as the effect size was not changed by adjustment for infection severity in the multivariable model (Figure 4) or by subgroup analyses stratified by infection severity. Attempts to link this to viral titers or viral shedding time were also unsuccessful. Participants who self-identified as White or Black race had higher IgG responses to Wuhan Hu-1 and B.1.1.7, but not to B.1.351, P.1, or B.1.617.2, compared with self-reported “other race.”

Finally, we examined the host characteristics associated with a positive response (OD value ≥0.3) against either Wuhan Hu-1 or B.1.1.7, plus either B.1.351 or P.1, to assess what factors were important in facilitating a robust cross-reactive response. Days since infection (>14 days vs ≤14 days), vaccination after infection, and higher disease severity were highly significant, whereas lower Adp:Lep was marginally associated (Supplementary Table

6). A similar association was noted when considering a positive response (OD value ≥0.3) against either Wuhan Hu-1 or B.1.617.2.

Overall, people with high levels of cross-reactive IgG following infection were more likely to have been vaccinated after infection, have had severe disease, have adipose tissue dysfunction, or self-identify as White or Black race. Other factors, including gender and immunocompromised status, were not associated with IgG levels against any of the variant RBDs tested.

Vaccination Increases Cross-reactive IgG Levels

Vaccination increased cross-reactive IgG levels in all populations. Younger people with healthy weight and more recent vaccination had the highest levels of cross-reactive IgG against all SARS-CoV-2 VOCs (Figure 5 and Supplementary Tables 7

Table 2. Risk Factors for Severe or Critical COVID-19

Characteristics	Nonsevere (n = 112)		Severe or Critical (n = 45)		Not Reported (n = 5)		P ^a
	n	(%)	n	(%)	n	(%)	
Gender							.29
Female	86	(76.8%)	38	(84.4%)	4	(80%)	
Male	26	(23.2%)	7	(15.6%)	1	(20%)	
Age, mean (SD), years	43.2 (12.0)		40.7 (11.0)		39.0 (4.1)		.32
Race (self-reported)							.36 ^b
White/Caucasian	88	(78.6%)	33	(73.3%)	4	(80%)	
Black/African American	13	(11.6%)	9	(20%)	0	(0%)	
Asian	6	(5.4%)	2	(4.4%)	1	(20%)	
Others	3	(2.7%)	1	(2.2%)	0	(0%)	
Not reported	2	(1.8%)	0	(0%)	0	(0%)	
Hispanic ethnicity	5	(4.5%)	1	(2.2%)	0	(0%)	.67
BMI classification							.0087 ^c
Underweight (<18.5 kg/m ²)	2	(1.8%)	0	(0%)	0	(0%)	
Normal (18.5 to <25.0 kg/m ²)	37	(33%)	8	(17.8%)	2	(40%)	
Overweight (25.0 to <30 kg/m ²)	32	(28.6%)	9	(20%)	1	(20%)	
Obese (30 to <40 kg/m ²)	32	(28.6%)	17	(37.8%)	0	(0%)	
Very obese (≥40 kg/m ²)	6	(5.4%)	7	(15.6%)	0	(0%)	
Not reported	3	(2.7%)	4	(8.9%)	2	(40%)	
Adiponectin to leptin ratio							.0436
Normal (≥1)	34	(30.4%)	8	(17.8%)	1	(20%)	
Abnormal (0.5 to <1)	18	(16.1%)	5	(11.1%)	2	(40%)	
Very abnormal (<0.5)	60	(53.6%)	32	(71.1%)	2	(40%)	
Immunocompromise	2	(1.8%)	5	(11.1%)	0	(0%)	.016
Patient contact							.88
None	52	(46.4%)	22	(48.9%)	5	(100%)	
Indirect	13	(11.6%)	6	(13.3%)	0	(0%)	
Direct	47	(42%)	17	(37.8%)	0	(0%)	

Participants who did not report were excluded from comparisons. Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; SD, standard deviation.

^aP value compares severe or critical vs asymptomatic or mild-moderate.

^bWhite/Caucasian vs others.

^cCompares obese and very obese vs not obese.

and 8). Specifically, after vaccination, obesity was associated with significantly lower levels of antibodies against RBD from Wuhan Hu-1 and B.1.1.7, but not B.1.351, P.1, or B.1.617.2, and time since vaccination and older age were both significantly associated with lower levels of antibody for all of the VOCs and cross-reactivity defined previously (Figure 5). Of interest, people with self-reported mild to moderate immunocompromise did not have reduced antibody responses to vaccines for any of the VOCs.

The host characteristics that resulted in lower VOC IgG responses postvaccination included obesity, time since vaccination, and older age (Figure 5). Further work is needed to define the mechanism(s) for the reduced IgG levels.

DISCUSSION

This study addresses a critical, unresolved question about IgG responses to SARS-CoV-2 infection and/or vaccination at an individual level—defining the characteristics associated with a lower cross-reactive IgG response against SARS-CoV-2 VOCs.

We determined that strong cross-reactive antibody responses after infection were positively associated with the following: 14 or more days since diagnosis, greater disease severity, and counterintuitively, poor metabolic health. Vaccination increased the magnitude and breadth of the antibody response, especially with past infection. However, in contrast to infection alone, the only other significant positive predictors of cross-reactive antibody response following vaccination were younger age and healthy BMI. This provides a snapshot of host characteristics associated with differential SARS-CoV-2 antibody responses. The results show the importance of vaccination and identify populations who have a high likelihood of requiring vaccine boosting to maintain protective antibody levels.

Strengths of the study include the cohort size; focus on hospital staff; detection of mild cases through regular screening with SARS-CoV-2 PCR; direct comparison between infection, vaccination, and vaccination postinfection; and statistical analysis to define the population least likely to mount broadly protective IgG responses [7, 8].

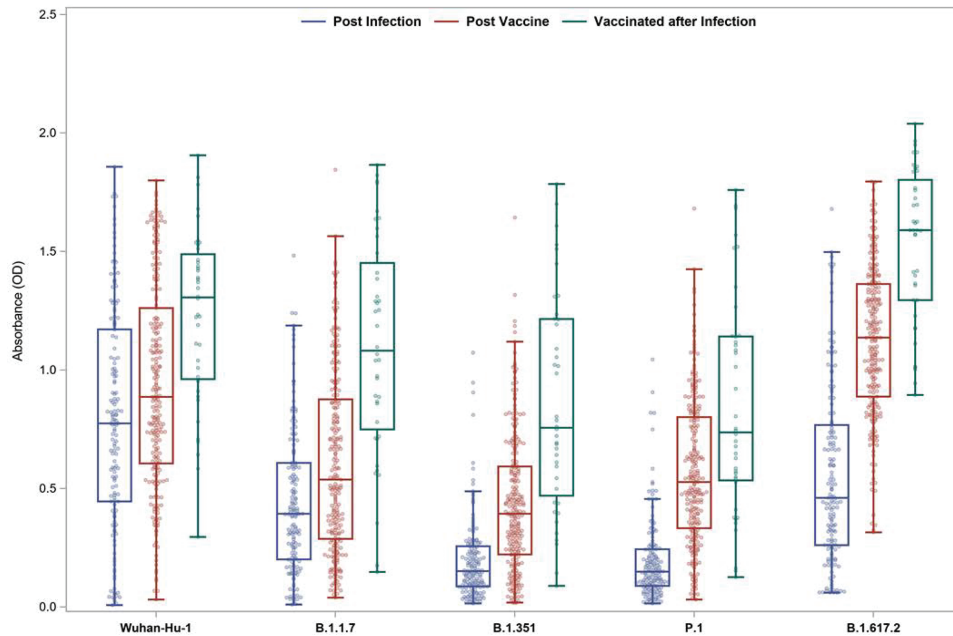


Figure 1. Receptor binding domain antibodies against SARS-CoV-2 variants following infection, vaccination, or vaccination after infection. The samples with a valid absorbance (OD) value for groups of participants who had SARS-CoV-2 infection, vaccination, or vaccination following infection are shown; 428 samples from 384 individuals collected >14 days and ≤90 days after the diagnosis or the final dose of the vaccine were included. The box plots with median, upper, and lower quartiles and minimum and maximum values for postinfection samples with an OD value from individuals who were infected, postvaccination samples from individuals who were only vaccinated, and postvaccination samples from individuals vaccinated after infection are shown in blue, red, and green, respectively. Abbreviations: OD, optical density; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

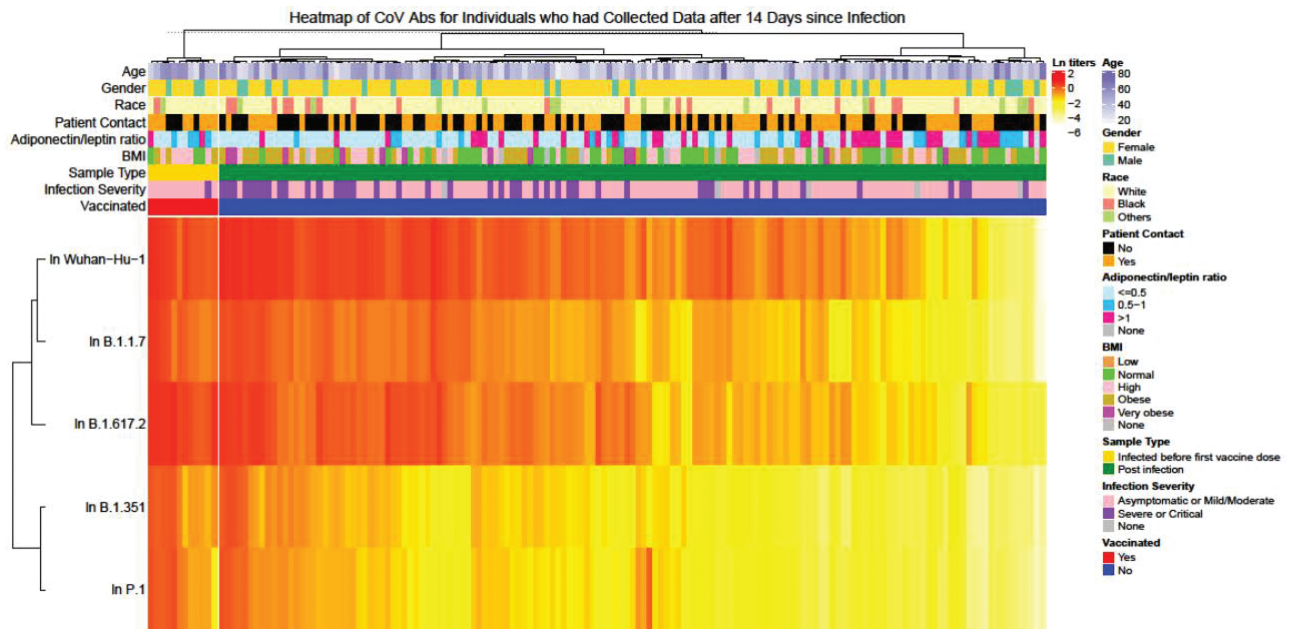


Figure 2. Heatmap of receptor binding domain antibody levels against SARS-CoV-2 variants after infection. Natural log-transformed OD values in all samples collected >14 days after a SARS-CoV-2 infection were clustered with Ward's hierarchical agglomerative clustering method using Spearman's correlation as the distance measure for transformed OD values and Euclidean distance for all covariates was applied. SARS-CoV-2 vaccination was used as a split variable in clustering. Abbreviations: Abs, antibodies; BMI, body mass index; CoV, coronavirus; In, natural log; OD, optical density; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

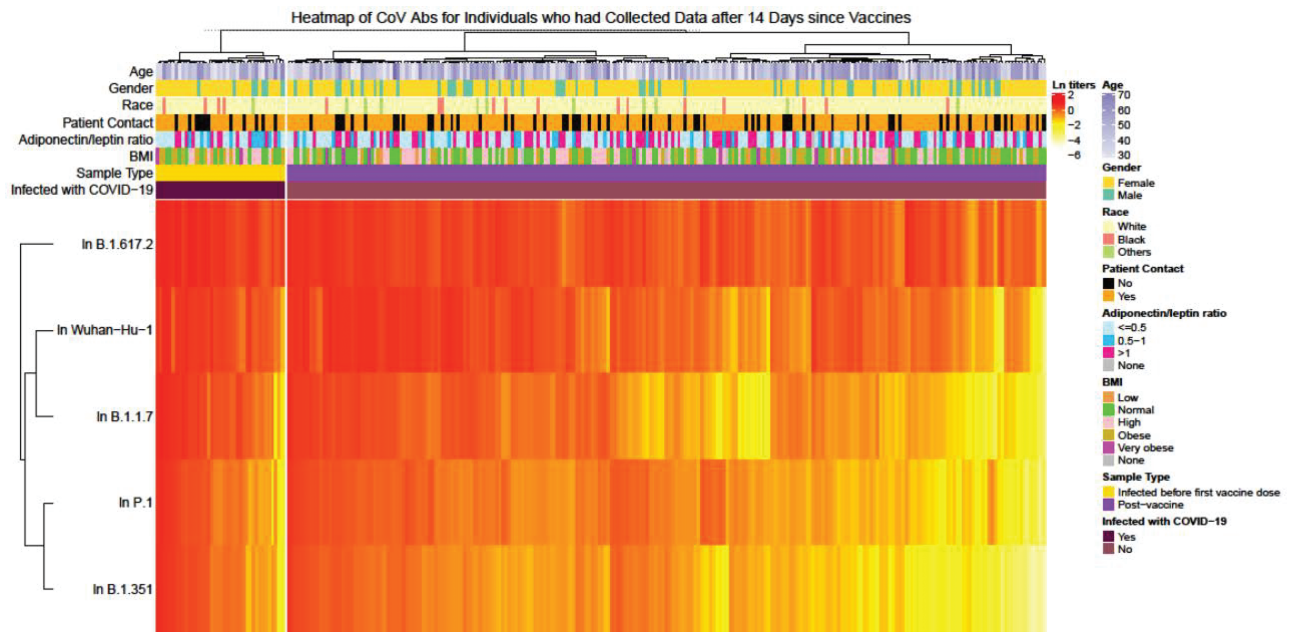


Figure 3. Heatmap of receptor binding domain antibody levels against SARS-CoV-2 variants after vaccination. Natural log-transformed OD values in all samples collected >14 days after SARS-CoV-2 vaccination were clustered with Ward's hierarchical agglomerative clustering method using Spearman's correlation as the distance measure for transformed OD values and Euclidean distance for all covariates was applied. SARS-CoV-2 infection was used as a split variable in clustering. Abbreviations: Abs, antibodies; BMI, body mass index; CoV, coronavirus; COVID-19, coronavirus disease 2019; ln, natural log; OD, optical density; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

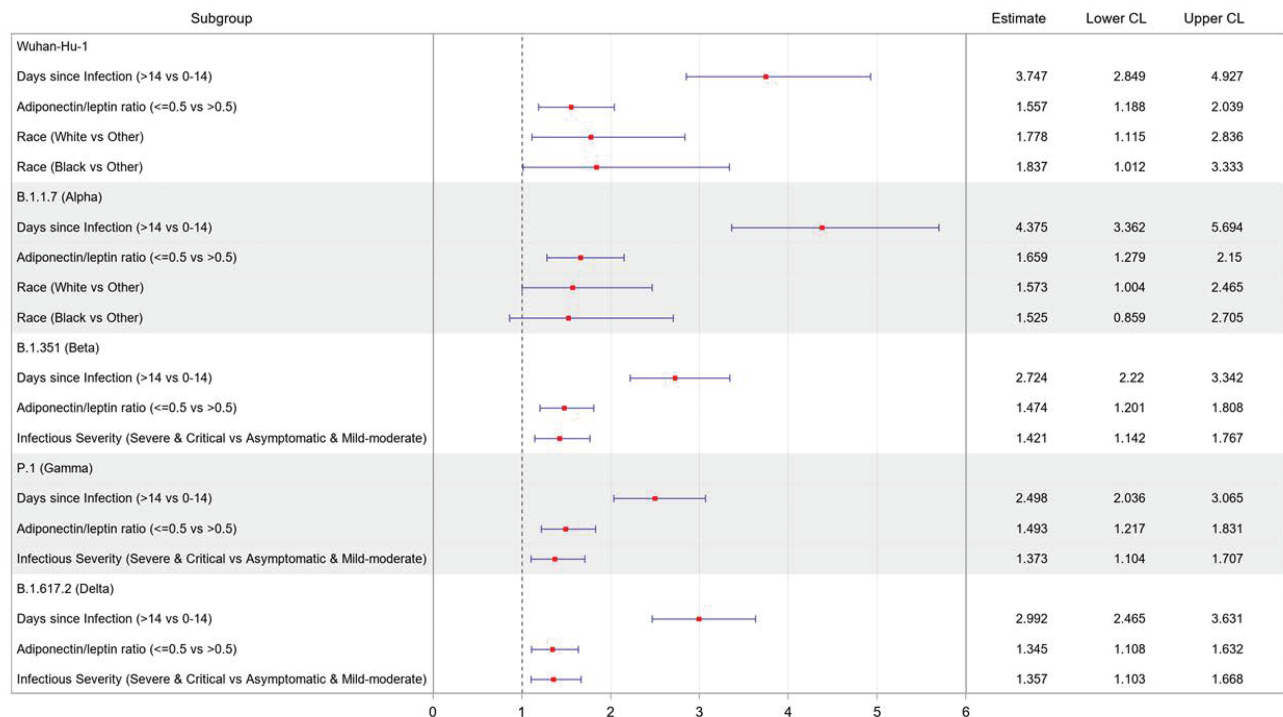


Figure 4. Multivariate analysis of predictors of receptor binding domain antibody levels against SARS-CoV-2 variants after infection. Multivariate linear models on natural log-transformed OD values were carried out among samples collected from 1 to 281 days after diagnosis with a SARS-CoV-2 infection without subsequent vaccination. Host characteristics, including gender, age, race, ethnicity, immunocompromised or not, BMI classification, adiponectin to leptin ratio, patient contact, time since infection diagnosis, infection severity, and time of infection (before or after 1 October 2020), were considered as covariate candidates. The elastic net model selection method was carried out with the BIC. The estimate was expressed in fold-change in the antibody level. Point estimates and 95% confidence intervals were transformed back to the original OD scale. Abbreviations: BIC, Bayesian information criterion; BMI, body mass index; CL, confidence limit; OD, optical density; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

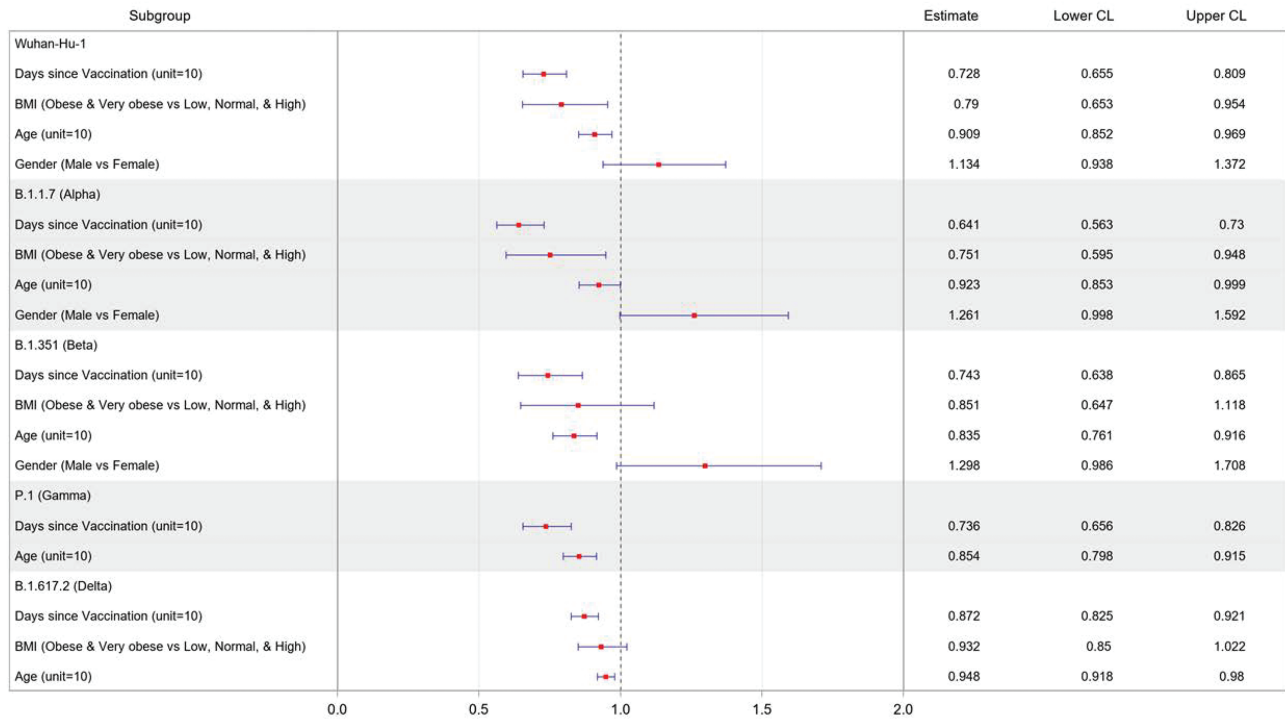


Figure 5. Multivariate analysis of predictors of receptor binding domain antibody levels against SARS-CoV-2 variants after vaccination. Multivariate linear models on natural log-transformed OD values were carried out among SARS-CoV-2-naïve participants with complete vaccination. Samples were collected 25 to 54 days after completion of vaccine course. Host characteristics, including gender, age, race, ethnicity, immunocompromised or not, BMI classification, adiponectin to leptin ratio, patient contact, and time since the final dose of the vaccine, were considered as covariate candidates. The elastic net model selection method was carried out with the BIC. The estimate was expressed in fold-change in the antibody level. Point estimates and 95% confidence intervals were transformed back to the original OD scale. Abbreviations: BIC, Bayesian information criterion; BMI, body mass index; CL, confidence limit; OD, optical density; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

The characteristics associated with the magnitude and breadth of IgG responses to SARS-CoV-2 VOCs differed between infection and vaccination. Following infection alone, we showed a clear association of IgG levels with disease severity and time since infection, which is in keeping with the literature [23, 24]. Published studies have shown that antibodies from late convalescence or following more severe disease exhibited increased neutralization potency to VOCs, suggesting persistence of cross-neutralizing plasma IgG levels. Age, gender, and immunocompromise had no significant effect on IgG levels after infection. If confirmed in other studies, the finding that moderate immunocompromise was not associated with decreased antibody responses to any of the variants would be reassuring for immunocompromised individuals. The lack of association between disease severity and gender was surprising since a male bias in COVID-19 mortality has been reported [25]. This may be due to the limited number of participants with severe disease. There was also no sex bias associated with overall difference in IgG responses, consistent with 1 other study [26]. Although we found an association between race and IgG levels, the construct is confounded by many social factors, so it is difficult to determine the relevance or mechanism of this finding.

We also demonstrated that high BMI or a low Adp:Lep, indicating adipose tissue dysfunction and metabolic syndrome, was associated with more severe disease, which parallels other studies [27, 28]. However, following infection only, poor metabolic health (Adp:Lep <0.5) was associated with higher IgG responses against VOCs. This was an independent factor unrelated to disease severity, and is consistent with other postconvalescent wild-type SARS-CoV-2 neutralizing antibody levels in individuals with metabolic syndrome comorbidities [29]. The reason for the association is unclear. Increased systemic inflammation caused by the metabolic syndrome might lead to higher antibody responses or, alternatively, these individuals might shed virus longer, leading to increased immune stimulation [30].

Vaccination resulted in plasma antibodies against VOCs that are mostly comparable to the anti-RBD ELISA titers against the original Wuhan Hu-1 strain. While the levels of cross-reactive IgG against RBD from multiple SARS-CoV-2 variants following vaccination were significantly associated with younger age, healthy weight, being a male, and more recent vaccination, vaccination improved IgG levels in all groups. Although the finding that being overweight was associated with lower antibody responses to most variants following vaccination is in

stark contrast to antibody levels following infection in participants with the metabolic syndrome, it is clearly consistent with other data suggesting that patients with obesity have poorer responses to other vaccines, such as influenza [31].

Overall, this study identifies factors influencing the magnitude and breadth of the IgG response to the spike RBD of 4 important SARS-CoV-2 VOCs. Limitations include the relatively low number of patients with critical illness or immunocompromised status, completion prior to widespread emergence of the Delta variant, and use of ELISA rather than neutralization titers. However, we have previously demonstrated that samples with an ELISA OD less than 0.3 have no neutralizing activity [12]. Further, this OD cutoff was defined in the original protocol as being the difference between samples that would be positive and negative against the spike protein [11]. These data could help prioritize vaccines and boosters for individuals expected to have the lowest antibody levels.

Conclusions

This study showed that vaccination improved IgG levels in all groups, highlighting the importance of vaccinating vulnerable populations, such as persons with poor metabolic health or high BMI, as well as those who had PCR-confirmed infection, to provide more broadly protective antibody responses to SARS-CoV-2 VOCs. However, older people and those with higher BMI are most likely to have lower antibody levels and could be prioritized for booster shots to retain broadly protective antibody levels. Overall, these studies highlight the importance of vaccinating vulnerable populations against COVID-19, including those with poor metabolic health or high BMI, immunocompromising conditions, and those who have previously had PCR-confirmed infection, to provide more broadly cross-reactive antibody responses to SARS-CoV-2 VOCs.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays (the "Serology Assays") and Newcastle disease virus-based SARS-CoV-2 vaccines which name F. K. as a co-inventor; F. K. also notes the following, which could be perceived as a conflict of interest: He has previously published work on influenza virus vaccines with S. Gilbert (University of Oxford); has consulted for Curevac, Merck, and Pfizer (before 2020); is currently consulting for Pfizer, Seqirus, and Avimex; his laboratory is collaborating with Pfizer on animal models of SARS-CoV-2; his laboratory is collaborating with N. Pardi at the University of Pennsylvania on mRNA vaccines against SARS-CoV-2; his laboratory was working in the past with GlaxoSmithKline on the development of influenza virus vaccines and 2 of his mentees have recently joined Moderna; F. K. reports royalties/licenses for Avimex; payment/honoraria for several academic lectures over the past 2 years. E. K. A. reports patents on methods for treating or reducing the severity of a viral infection. J. H. E. reports grants/support to their institution from the following: Pfizer, Eli Lilly and Company, Forma Therapeutics, Global Blood Therapeutics, National Institutes of Health, and American Society of Hematology; reports personal consulting fees from Global Blood Therapeutics, Emmaus Life Sciences, Daiichi Sankyo, and Agios; personal payments/honoraria for educational events from Global Blood Therapeutics; personal payments from the National Institutes of Health for serving on a Data Safety and Monitoring Board/Advisory Board; receipt of medical writing support from Global Blood Therapeutics. P. G. T. reports the following funding: NIAID 5R01AI128805-05, 1R01AI154470, 75N93021C00016, 75N93021C00018, 5R01AI121832, 5R01AI136514, and 5R01AI35025; reports personal consulting fees from Johnson and Johnson, Cytoagents, and Immunoscape; reports personal payments for educational events from Illumina-Future Genomic Advances, Yale University, CZ Biohub, PACT Pharma, UCSD, Tufts, University of Arizona, Mt. Sinai, UMass, OSU, Korean Association of Immunology, SISMID, University of Washington, MSKCC, Washington University, University of Missouri, Fred Hutch, UNM, Illumina Single Cell, and Cincinnati Childrens; reports travel support for the following: NIH Study Section, Midsummer Immunology, NIAID CEIRR, CEIRS, and CIVIC meetings; GRC, 10X Users Symposium, Illumina Symposia; reports the following licenses/patents: Methods for Treating or Reducing the Severity of a Viral Infection, US20170304293A1 Coordinated metabolic reprogramming in response to productive viral infections, and Cloning and expression system for T-cell receptors (license payments made to them and their institution). S. S.-C. reports the following support: NIAID 5R01AI125392-05, 5U01AI144616-03S1, 5R01AI140766-03, 5R01AI123144-05, 75N93021C00016, 75N93021C00018, 3R21AI135254-02S1, and 5R21AI135254-02; consulting fees from CIDARA (personal fees), Versatope, and GenoTwin; payments/honoraria for educational activities for American Philosophical Society (paid to University of Wisconsin), NIAID workshops (several; paid to Wayne State), NEDL/Boston University (paid to the University of Nebraska); travel support for the following events: GRC, NIH Study Section, ASM PSAC, and NIAID CEIRR, CEIRS, and CIVIC meetings; participation in DMB 16-0092; and serving as Chair, PSAC, for the American Society for Microbiology. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of

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St. Jude Investigative Team. Department of Biostatistics, St. Jude Children's Research Hospital, Memphis, Tennessee, USA: Tomi Mori; Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee, USA: Diego R. Hijano, Hana Hakim, Ronald H. Dallas, Valerie Cortez, Ana Vazquez-Pagan, Richard J. Webby, Thomas Fabrizio, Jamie Russell-Bell; Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA: David C. Brice, Ashley Castellaw, Resha Bajracharya, Brandi L. Clark, Lee-Ann Van de Velde, Walid Awad, Taylor L. Wilson, Allison M. Kirk; Department of Hematology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA: Jason Hodges; Department of Global Pediatric Medicine, St. Jude Children's Research Hospital, Memphis, Tennessee, USA: James Sparks; Office of Quality and Patient Care, St. Jude Children's Research Hospital, Memphis, Tennessee, USA: David E. Wittman; Department of Pathology, St. Jude Children's Research Hospital, Memphis, Tennessee, USA: Randall T. Hayden; Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee, USA: James Hoffman.

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