Understanding ‘hybrid immunity’: comparison and predictors of humoral immune responses to SARS-CoV-2 infection and COVID-19 vaccines

Nusrat J. Epsi¹,², Stephanie A. Richard¹,², David A. Lindholm³,¹⁰, Katrin Mende¹,²,³, Anuradha Ganesan¹,²,⁴, Nikhil Huprikar⁴, Tahaniyat Lalani¹,²,⁵, Anthony C. Fries⁶, Ryan C. Maves¹,⁷, Rhonda E. Colombo¹,²,⁸,¹⁰, Derek T. Larson⁹,¹³, Alfred Smith⁵, Sharon W. Chi¹,²,¹⁰, Carlos J. Maldonado¹¹, Evan C. Ewers⁹, Milissa U. Jones¹², Catherine M. Berjohn¹,¹⁰,¹³, Daniel H. Libraty¹,¹³, Margaret Sanchez Edwards¹,², Caroline English¹,², Julia S. Rozman¹,², Rupal M. Mody¹⁴, Christopher J. Colombo⁸,¹⁰, Emily C. Samuels¹⁵, Princess Nwachukwu¹⁵, Marana S. Tso¹⁵, Ann I. Scher¹⁰, Celia Byrne¹⁰, Jennifer Rusiecki¹⁰, Mark P. Simons¹, David Tribble¹, Christopher C. Broder¹⁵, Brian K. Agan¹,², Timothy H. Burgess¹, Eric D. Laing¹⁵, Simon D. Pollett*¹,² for the EPICC COVID-19 Cohort Study Group**

** EPICC COVID-19 Cohort Study Group members are listed in the Acknowledgments section.

¹Infectious Disease Clinical Research Program, Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

²Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD, USA

³Brooke Army Medical Center, Fort Sam Houston, TX, USA

⁴Walter Reed National Military Medical Center, Bethesda, MD, USA

© The Author(s) 2022. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com This article is published and distributed under the terms of the Oxford University Press, Standard Journals Publication Model (https://academic.oup.com/journals/pages/open_access/funder_policies/chorus/standard_publication_model)
Corresponding Author:

Simon Pollett, MBBS
6720A Rockledge Drive, Suite 250
Bethesda, MD 20817
spollett@idcrp.org

Short Title: Hybrid Immunity: Infection and Vaccines
ABSTRACT:

Background: Comparing humoral responses in SARS-CoV-2 vaccinees, those with SARS-CoV-2 infection, or combinations of vaccine/infection (‘hybrid immunity’), may clarify predictors of vaccine immunogenicity.

Methods: We studied 2660 U.S. Military Health System beneficiaries with a history of SARS-CoV-2 infection-alone (n = 705), vaccination-alone (n = 932), vaccine-after-infection (n = 869), and vaccine-breakthrough-infection (n = 154). Peak anti-spike-IgG responses through 183 days were compared, with adjustment for vaccine product, demography, and comorbidities. We excluded those with evidence of clinical or sub-clinical SARS-CoV-2 reinfection from all groups.

Results: Multivariable regression results indicated vaccine-after-infection anti-spike-IgG responses were higher than infection-alone (p < 0.01), regardless of prior infection severity. An increased time between infection and vaccination was associated with a greater post-vaccination IgG response (p < 0.01). Vaccination-alone elicited a greater IgG response, but more rapid waning of IgG (p < 0.01), compared to infection-alone (p < 0.01). BNT162b2 and mRNA-1273 vaccine-receipt was associated with greater IgG responses compared to JNJ-78436735 (p < 0.01), regardless of infection history. Those with vaccine-after-infection or vaccine-breakthrough-infection had a more durable anti-spike-IgG response compared to infection-alone (p < 0.01).

Conclusions: Vaccine-receipt elicited higher anti-spike-IgG responses than infection-alone, although IgG levels waned faster in those vaccinated (compared to infection-alone). Vaccine-after-infection elicits a greater humoral response compared to vaccine or infection alone; and the timing, but not disease severity, of prior infection predicted these post-vaccination IgG responses. While differences between groups were small in magnitude, these results offer insights into vaccine immunogenicity variations that may help inform vaccination timing strategies.

Key words: SARS-CoV-2, IgG, antibody response, vaccine, vaccine breakthrough
INTRODUCTION

Coronavirus disease 2019 (COVID-19) mRNA vaccines induce severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein expression in vaccine recipients resulting in robust humoral and T-cell responses [1]. In the United States, two mRNA vaccines were authorized in December 2020 [2-4]. Since their widespread use in the United States, and elsewhere, these two mRNA vaccines proved highly effective in preventing, hospitalization, morbidity, and death [5-7]. The US Food and Drug Administration (FDA) also authorized use of a viral vectored vaccine using an adenovirus-26 backbone in February 2021 [8].

While there is strong evidence of COVID-19 vaccines’ effectiveness and safety [2, 3, 6, 9, 10], full characterization of durability of vaccine-induced humoral immunity and predictors of a durable vaccine immune response remains important to investigate. Comparing humoral immune responses of SARS-CoV-2 vaccine recipients with and without a history of SARS-CoV-2 infection, to immunity induced by SARS-CoV-2 infection-alone, helps to identify such predictors of vaccine immune response and potentially inform vaccination timing strategies.

Prior studies noted that infection-induced and vaccine-induced immunity were durable for at least six-months, but those vaccinated with prior infection (i.e., vaccine-after-infection) mounted a greater and more durable level of humoral response [11-13]. While these studies emphasized the value of vaccination following a prior infection, additional data replicating these findings add to consensus for vaccination regardless of infection history. However, several questions surround SARS-CoV-2 ‘hybrid immunity’ induced by infection and subsequent vaccination. It is unclear whether initial severity of illness or time-between-infection and subsequent vaccination modifies this post-infection vaccine IgG ‘boost’. The emergence of Omicron variant underscores importance of understanding predictors of durable and robust
vaccine immunity, particularly as a rapidly growing proportion of the population with a history of vaccination before or after infection develops ‘hybrid immunity’.

We sought to (i) compare magnitude and durability of vaccine-induced humoral immunity against humoral immune response elicited by SARS-CoV-2 infection, adjusting for vaccine product and vaccine recipient baseline health and age. We then (ii) confirmed whether vaccine-induced immunity following prior infection (‘hybrid immunity’) offered more robust humoral response than afforded by prior infection/vaccination alone, and (iii) whether this was affected by either host characteristics (age and comorbidities), severity or timing of the prior infection, specific product vaccine received, or number of vaccine doses received.

METHODS

Study population and general study design:

The Epidemiology, Immunology, and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC) study is a longitudinal cohort study of U.S. Military Health System (MHS) beneficiaries with a history of SARS-CoV-2 infection and/or vaccinations (see Supplementary Material for extended details). Briefly, eligibility criteria for enrollment include those MHS beneficiaries presenting to a military treatment facility (MTF) for SARS-CoV-2 polymerase chain reaction (PCR) testing. In early 2021, eligibility for EPICC was expanded to enroll those receiving an FDA authorized SARS-CoV-2 vaccine i.e., BNT162b2 (Pfizer/BioNTech), mRNA-1273 (Moderna), and JNJ-78436735 (Janssen) within the MHS, with enrollment on day of vaccination.

EPICC enrollment occurs at ten MTFs: Brooke Army Medical Center, Fort Belvoir Community Hospital, Madigan Army Medical Center, Naval Medical Center Portsmouth, Naval Medical Center San Diego, Tripler Army Medical Center, Walter Reed National Military
Medical Center, Carl R. Darnall Army Medical Center, William Beaumont Army Medical Center, and Womack Army Medical Center. To permit scalability of enrollment across other geographic areas (including non-US locations), and to enhance convenience to subjects, beginning on October 19th, 2020 MHS beneficiaries who were either tested for SARS-CoV-2 and/or vaccinated were also eligible for enrollment and followed remotely via an online pathway. (Supplementary Table 1).

The EPICC study therefore enrolls and follows participants who are SARS-CoV-2 test positive, SARS-CoV-2 test negative, and those vaccinated with or without a history of SARS-CoV-2 infection. MHS beneficiaries enrolled in EPICC who were diagnosed with SARS-CoV-2 and/or vaccinated against SARS-CoV-2 from March 20, 2020 through November 15, 2021 were included in this analysis.

We excluded from the analysis those participants who tested SARS-CoV-2 negative and had not received any vaccination. We also excluded those who received vaccine as part of a clinical trial. In addition, children (age <18 years) were excluded from the analysis due to later implementation of vaccines in pediatric populations (Supplementary Figure 1).

**Diagnosis of SARS-CoV-2 infection and case definition:**

SARS-CoV-2 infection was determined by positive PCR clinical laboratory test performed at the enrolling MTF or follow-up positive upper respiratory swab collected as part of the EPICC study by day 17 post-enrollment and tested by PCR [2], and/or supplemented with self-reported testing history in online-enrolled participants (see Supplementary Materials).

**Ascertainment and definitions of COVID-19 vaccine history**

Vaccination status was established, including the date and product of vaccine receipt, through MDR in addition to CRF and supplemented by questionnaire self-report (see
Supplementary Materials). We defined “completed primary vaccine-series” by 14-days after (a) a 2-dose series of mRNA vaccine administered 21-days (i.e., BNT162b2) or 28-days (i.e., mRNA-1273) apart or (b) a single-dose of viral vector vaccine (i.e., JNJ-78436735).

*Measurement of SARS-CoV-2 anti-spike-IgG response*

Anti-spike immunoglobulin G (IgG) responses were measured by multiplex microsphere-based immunoassay, described in detail previously [14, 15], which has been used in concurrent testing with the Mt. Sinai EUA ELISA and performed with 99% concordance [16] (see Supplementary Materials). Antigen-specific SARS-CoV-2 spike and NP reactive IgG levels were reported as a median fluorescence intensity (MFI).

*Comparisons of anti-spike-IgG responses to SARS-CoV-2 infection and vaccines*

The primary outcome of interest in this analysis was peak observed SARS-CoV-2 anti-spike-IgG MFI assessed out to 183-days post vaccine/infection exposure (i.e., final vaccine date or symptom onset date, respectively). This period was constrained by the duration of post-vaccine follow-up time available. Log₁₀ transformations were applied to anti-spike-IgG MFI values to normalize the data and exponentiated coefficients were used to interpret throughout the paper. We compared four groups for anti-spike-IgG responses to SARS-CoV-2 infection and/or vaccines: (i) “infection-alone” (those who tested SARS-CoV-2 positive and did not receive any subsequent vaccination), (ii) “vaccination-alone” (completed primary vaccination-series, without a known history of SARS-CoV-2 infection), (iii) “vaccination-after-infection” (those who tested SARS-CoV-2 positive and then received complete primary vaccination-series), and (iv) “vaccine-breakthrough-infection” (those who were infected by SARS-CoV-2 two or more weeks after complete primary vaccination-series). Given the small numbers of subjects who received booster doses and limited follow-up time, these participants were excluded from analysis. We
retained subset of participants classified as ‘vaccine-after-infection’ who received only one-dose
of mRNA vaccine (compared to two-doses) to examine humoral immune response (see
Supplementary Material for extended details).

Adjusted comparisons of anti-spike-IgG responses to SARS-CoV-2 infection and vaccines and
identification of predictors of humoral immune response

We performed unadjusted and adjusted comparisons of peak observed SARS-CoV-2 anti-
spike-IgG MFI between four-groups using univariable and multivariable linear regression. These
models considered other potential predictors of vaccine and/or infection humoral response,
including: serum specimen sampling time i.e., time between date of serum specimen collection
of peak anti-spike-IgG and date of final dose of vaccine receipt or SARS-CoV-2 infection
symptom onset date (whichever was latest), comorbidities, sex, age, and vaccine product
received. We examined post-vaccination anti-spike-IgG response in subjects with a prior
infection history with multivariable regression model to examine how severity of initial infection
changed peak post-vaccination anti-spike-IgG. We conducted Spearman correlation (\( \rho \)) to
explore whether increasing time-from-infection-to-vaccination correlated with increased peak
post-vaccination IgG.

We used Charlson Comorbidity Index (CCI) [17] to quantify comorbidities measured in MDR or
self-reported by subject; age, sex, race, and ethnicity were reported by the participant. We
categorized infection severity by the need for hospitalization for COVID-19. Descriptive
statistics were calculated for demographic characteristics, comorbidities, and illness severity by
four-groups, with \( P \)-values computed using Fisher’s exact test. Non-parametric Mann-Whitney U
test and Kruskal-Wallis variance analysis were used to evaluate the data. Figures were generated
and statistical analyses were performed in RStudio version 4·0·2 software [18].
Repeated measures sensitivity analysis:

We extended above analyses (which used a single timepoint anti-spike-IgG value with adjustment for sampling time after latest antigenic exposure) and confirmed findings with a repeated measures model which incorporated within-subject changes in anti-spike-IgG over time and restricted to those with two or more sera specimens collected. For this mixed linear model, we used anti-spike-IgG as dependent variable while controlling for random-effects of subjects and fixed-effect of time-since-infection-to-vaccination. The coefficients derived from this estimate indicated whether vaccination-alone, vaccine-after-infection, and vaccine-breakthrough-infection were associated with larger peak IgG response compared to SARS-CoV-2 infection-alone. We fit an interaction term of sampling time by group to estimate whether IgG quantity waned faster by group.

Ethics

This study was approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Review Board (IRB) under protocol IDCRP-085; all participants or their legally authorized representative provided informed consent to participate.

RESULTS

Demographic, clinical, and antigenic exposure characteristics of EPICC participants

Among 3475 SARS-CoV-2 tested and/or vaccinated participants who were enrolled in EPICC, 2660 (84%) had sera or dried blood spot (DBS) collected and were included in this analysis: SARS-CoV-2 infection-alone (n = 705), SARS-CoV-2 vaccinated-alone (n = 932), vaccine-after-infection (n = 869), and vaccine-breakthrough-infection (n = 154) (Supplementary Figure 1). This total number of subjects included 189 and 217 participants who received one-dose of vaccine in vaccination-alone and vaccine-after-infection group respectively, at the time
of data cutoff for analysis; these subjects were not used for four-group comparison but were used for supplemental analyses. This analysis set excluded (n = 209) clinically apparent infections and (n = 502) subclinical reinfections across all four-groups (Supplementary Figure 1). Most (79.9\%) vaccinee received BNT162b2 vaccine, 18.9\% of vaccinee received mRNA-1273 vaccine, and 1.2\% of vaccinee received JNJ-78436735.

More than half of these subjects were male (59.4\%), 66.2\% were 18-44 years old, and 81\% had no comorbidities at enrollment (Table 1). The median age of these enrolled participants was 38 years (IQR: 20.7). The median sampling time for each group was evaluated based on latest antigenic exposure (SARS-CoV-2 infection/vaccination) and is presented in Table 1.

Unadjusted and adjusted comparison of anti-spike-IgG response to SARS-CoV-2 infection and/or vaccination

The observed maximum (peak) SARS-CoV-2 anti-spike-IgG by sampling time are presented in Figure 1. The mean unadjusted peak anti-spike-IgG level was higher in vaccine-after-infection subjects compared to infection-alone or vaccination-alone (p < 0.01), and highest in those with vaccine-breakthrough-infection (Figure 2). Age group-stratification revealed similar findings related to peak anti-spike-IgG responses (Supplementary Figure 2A). Even after a single-dose of mRNA-vaccine following prior infection, peak anti-spike-IgG response was greater than peak anti-spike-IgG responses after infection-alone (p < 0.01; Supplementary Figure 2B).

To account for differences in post-infection/post-vaccine sera sampling time and host characteristics between groups (Table 1), we examined for differences in peak anti-spike-IgG between groups and demonstrated that vaccination-alone status was associated with 1.07 MFI increase in peak anti-spike-IgG, compared to those with SARS-CoV-2 infection-alone, after
adjusting for host characteristics (i.e., age, sex, CCI and sampling time) (p < 0.01; Table 2). We confirmed that SARS-CoV-2 vaccination-after-infection is associated with a larger peak anti-spike-IgG response (increase of 1.13 MFI compared to SARS-CoV-2 infection-alone, adjusting for group, and host characteristics; p < 0.01; Table 2), and vaccine-breakthrough-infection is associated with highest peak anti-spike-IgG (1.2 MFI, p <0.01, Table 2).

Comparison of vaccine product responses, accommodating infection history and host characteristics

When stratified by each vaccine product, we consistently noted highest median anti-spike-IgG response in vaccine-breakthrough-infection group (Supplementary Figure 2C). The vaccination-after-infection group had a higher peak anti-spike-IgG response compared to the vaccination-alone group (Supplementary Figure 2C).

When directly compared between vaccine types (unadjusted), a receipt of mRNA vaccines (BNT162b2, mRNA-1273) induced a higher peak anti-spike-IgG compared with JNJ-78436735 (p < 0.01, Supplementary Figure 2D). Even after adjusting for differences in host characteristics, and prior infection history between vaccine recipient groups JNJ-78436735 receipt produced lower peak response compared to BNT162b2 and mRNA-1273 groups (Table 3). These adjusted comparisons also noted statistically significant difference in magnitude of peak responses between BNT162b2 and mRNA-1273, but magnitude of this difference was small (Table 3, Supplementary Table 2).

Correlation of severity and timing of initial infection with post-vaccine anti-spike-IgG response

We examined predictors of vaccine-after-infection anti-spike-IgG responses, specifically whether this was dependent on severity of initial infection. We fit a multivariable regression model restricted to vaccine-after-infection subjects (Table 4). The result indicated that being
hospitalized for COVID-19 (compared to outpatient COVID-19 infection history) was not
associated with higher observed post-vaccine peak anti-spike-IgG \( \log_{10} \) (\( p = 0.88 \)). However, we
noted an increasing post-vaccine IgG peak with increasing time-since-infection-to-vaccination
(Supplementary Figure 3).

Estimation of anti-spike-IgG waning rates by vaccination and/or infection

A mixed effects model derived from longitudinally collected sera compared within-
subject changes of anti-spike-IgG levels among groups. The findings confirmed prior single-
timepoint analyses, which were: vaccination-alone was associated with a higher peak IgG than
infection-alone; vaccine-breakthrough-infection was associated with highest peak spike IgG
response, and vaccination-after-infection was associated with a higher peak anti-spike-IgG
response than vaccine-alone or infection-alone (Supplementary Table 3).

To better understand anti-spike-IgG durability, we included a regression model interaction term
of vaccine/infection history and sampling time to specifically estimate the rate of anti-spike-IgG
waning. These longitudinal time-varying interaction coefficients permitted valid estimation of
waning rates which weighted moving averages of cross-sectional data (Figure 1) could not
provide. The coefficient estimates indicated that anti-spike-IgG waned faster in those who just
received a vaccine compared to SARS-CoV-2 infection (Table 5, Figure 3). Post-vaccination
anti-spike-IgG waned slowest in those with vaccine-breakthrough-infection and those vaccine-
after-infection (Table 5, Figure 3). We performed two sensitivity analyses: (i) removing
potentially influential datapoints and compared standardized differences between regression
coefficients (using \textit{dfbeta} command); (ii) restricting to non-severe (i.e., outpatients) SARS-CoV-
2 infections. The findings from both sensitivity analyses were similar to the overall analysis
(Supplementary Table 4-5).
DISCUSSION

Our findings confirm that ‘hybrid immunity’ arising from COVID-19 vaccine-induced immunity after prior infection offers greater humoral immunogenicity than either vaccination/prior infection-alone. Furthermore, humoral immune response to prior infection is substantially augmented by even a single-dose of vaccine, corroborating other studies [19-24]. These findings are particularly relevant as the Delta and Omicron variants have led to a large proportion of the population with ‘hybrid immunity’. Our findings expand upon prior studies on this topic and showing that increasing infection-to-vaccine-time, but not initial infection severity, correlates with a greater post-vaccination response. This finding may help guide strategies on timing of mRNA vaccine dosing after initial SARS-CoV-2 infection, although this requires further study. In addition, we noted that vaccine-breakthrough-infections offer highest peak IgG response (even as breakthrough-infections were noted to be milder than pre-vaccine infections), emphasizing that repeated exposures to SARS-CoV-2 antigens lead to higher IgG responses. This latter finding is consistent with recent data showing mRNA vaccine booster doses lead to significant increases in immunogenicity [25]. Importantly, these analyses only refer to antibody kinetics and do not measure cellular immune response or vaccine effectiveness directly.

Our results indicate that receipt of mRNA vaccines (in those without a history of infection) offer higher short-to-medium term peak humoral responses compared to SARS-CoV-2 infection-alone, after adjustment for other predictors (Table 2). However, we noted that vaccine-alone group had more rapid waning of IgG compared to those with SARS-CoV-2 infection-alone and those with vaccine-after-infection. The difference waning rates between groups was small, and their clinical significance unclear [26]. These data contribute to the discussion about significance of recent comparisons of infection frequency and clinical outcomes among vaccinees when
compared to infection-alone subjects, and those with a history of both infection and vaccination [27-32]. These findings also contribute to our understanding of how vaccine-induced immunity compares to SARS-CoV-2 infection and optimal timing of vaccine boosting.

Our study also permitted a head-to-head comparison of COVID-19 vaccines. As with other studies, we noted a greater peak anti-spike-IgG response in those who received an mRNA vaccine (mRNA-1273, BNT162b2) compared to JNJ-78436735 vaccine when adjusting for host characteristics and infection history. Among mRNA vaccines, we also noted that mRNA-1273 vaccine showed a higher peak IgG compared to BNT162b2 (Table 3, Supplemental Table S2) [33-35]. This is consistent with published findings that shows a greater humoral immune response from mRNA-1273 and –in some studies –apparent increased effectiveness of the mRNA-1273 vaccine.

This analysis has several limitations. First, these results refer only to binding antibody assays due to prohibitive logistics for performing neutralizing antibody assays on these many specimens. However, this binding assay (and other binding anti-spike-IgG assays) has moderate-to-high correlation with neutralizing antibody titers previously performed on a small number of EPICC subjects [15]. Second, quantitative differences of anti-spike-IgG binding responses, measured as MFI at a single blood specimen dilution (1:400) between groups were overall small, which may reflect upper limits of assay quantification, and anti-spike-IgG MFI saturation in blood samples collected after multiple immunogen stimulations. Third, follow-up time did not exceed 183-days, yet the study spanned a period of sequential dominant variants in the United States. Finally, our understanding on how antibody responses serve as a correlate of clinical protection is still developing [36]. While a landmark meta-analysis estimated a neutralizing antibody titer which could protect against infection [37], defining a protective titer remains challenging and is subject
to assay-to-assay comparability and the particular clinical endpoint of interest. Further, there is increasing evidence on the protective role of T-cell immune responses against infection and severity, further complicating estimations of a protective antibody titer [38, 39]. These limitations are important to acknowledge before inference is undertaken of relative protection between those with different antigenic exposure histories. They also underscore need for further study in this and other cohorts, to include integration of clinical outcomes with a range of immune readout data from B-cell, T-cell, and innate arms of immune system, as well as in pediatric age groups and booster recipients which were not part of this analysis.

The strengths of this study included a large sample size, measurement of a broad range of subject and illness characteristics to account for confounding between four groups, and careful ascertainment of subclinical repeat infections that could have biased comparisons. Taken together, these findings provide further data on importance of antiviral immunogenicity by vaccination beyond that afforded by prior SARS-CoV-2 infection and offer further insights into host responses to sequential SARS-CoV-2 antigenic exposure which may inform future vaccination strategies and their development, including evaluation on timing of post-infection vaccine administration.

NOTES

ACKNOWLEDGEMENTS

We sincerely thank the members of the EPICC COVID-19 Cohort Study Group for their many contributions in conducting the study and ensuring effective protocol operations. The authors wish to also acknowledge all who have contributed to the EPICC COVID-19 study:

Brooke Army Medical Center, Fort Sam Houston, TX: Col J. Cowden; LTC M. Darling; S. DeLeon; Maj D. Lindholm; LTC A. Markelz; K. Mende; S. Merritt; T. Merritt; LTC N. Turner; CPT T. Wellington

Carl R. Darnall Army Medical Center, Fort Hood, TX: LTC S. Bazan; P.K Love
Fort Belvoir Community Hospital, Fort Belvoir, VA: N. Dimascio-Johnson; MAJ E. Ewers; LCDR K. Gallagher; LCDR D. Larson; A. Rutt

Henry M. Jackson Foundation, Inc., Bethesda, MD: P. Blair; J. Chenoweth; D. Clark

Madigan Army Medical Center, Joint Base Lewis McChord, WA: S. Chambers; LTC C. Colombo; R. Colombo; CAPT C. Conlon; CAPT K. Everson; COL P. Faestel; COL T. Ferguson; MAJ L. Gordon; LTC S. Grogan; CAPT S. Lis; COL C. Mount; LTC D. Musfeldt; CPT D. Odineal; LTC M. Perreault; W. Robb-McGrath; MAJ R. Sainato; C. Schofield; COL C. Skinner; M. Stein; MAJ M. Switzer; MAJ M. Timlin; MAJ S. Wood

Naval Medical Center Portsmouth, Portsmouth, VA: S. Banks; R. Carpenter; L. Kim; CAPT K. Kronmann; T. Lalani; LCDR T. Lee; LCDR A. Smith; R. Smith; R. Tant; T. Warkentien

Naval Medical Center San Diego, San Diego, CA: CDR C. Berjohn; S. Cammarata; N. Kirkland; D. Libraty; CAPT (Ret) R. Maves; CAPT (Ret) G. Utz

Tripler Army Medical Center, Honolulu, HI: S. Chi; LTC R. Flanagan; MAJ M. Jones; C. Lucas; LTC (Ret) C. Madar; K. Miyasato; C. Uyehara

Uniformed Services University of the Health Sciences, Bethesda, MD: B. Agan; L. Andronescu; A. Austin; C. Broder; CAPT T. Burgess; C. Byrne; COL (Ret.) K Chung; J. Davies; C. English; N. Epsi; C. Fox; M. Fritschlanski; M. Gruber; A. Hadley; COL P. Hickey; E. Laing; LTC C. Lanteri; LTC J. Livezey; A. Malloy; R. Mohammed; C. Morales; P. Nwachukwu; C. Olsen; E. Parmeelee; S. Pollett; S. Richard; J. Rozman; J. Rusiecki; E. Samuels; P. Nwachukwu; M. Tso; M. Sanchez; A. Scher; CDR M. Simons; A. Snow; K. Telu; D. Tribble; L. Ulomi

United States Air Force School of Aerospace Medicine, Dayton, OH: TSgt T. Chao; R. Chapleau; M. Christian; A. Fries; C. Harrington; V. Hogan; S. Huntsberger; K. Lanter; E. Macias; J. Meyer; S. Purves; K. Reynolds; J. Rodriguez; C. Starr

United States Coast Guard, Washington, DC: CAPT J. Iskander, CDR I. Kamara

Womack Army Medical Center, Fort Bragg, NC: B. Barton; LTC D. Hostler; LTC J. Hostler; MAJ K. Lago; C. Maldonado; J. Mehrer

William Beaumont Army Medical Center, El Paso, TX: MAJ T. Hunter; J. Mejia; J. Montes; R. Mody; R. Resendez; P. Sandoval; M. Wayman

Walter Reed National Military Medical Center, Bethesda, MD: I. Barahona; A. Baya; A. Ganesan; MAJ N. Huprikar; B. Johnson

Walter Reed Army Institute of Research, Silver Spring, MD: S. Peel

GROUP AUTHORS:

We thank the members of the EPICC COVID-19 Cohort Study Group for their many contributions in conducting the study and ensuring effective protocol operations. The following
members were all closely involved with the design, implementation, and oversight of the study and have met group authorship criteria for this manuscript:

Brooke Army Medical Center, Fort Sam Houston, TX: LTC M. Darling; T. Merritt; CPT T. Wellington

Fort Belvoir Community Hospital, Fort Belvoir, VA: N. Dimascio-Johnson;

ACESO, Henry M. Jackson Foundation, Inc., Bethesda, MD: J. Chenoweth; D. Clark; P. Blair

Madigan Army Medical Center, Joint Base Lewis McChord, WA: S. Chambers; R. Colombo; COL P. Faestel; CPT S. Lis; CPT D. Odineal; LTC M. Perreault; C. Schofield; M. Stein

Naval Medical Center San Diego, San Diego, CA: CDR C. Berjohn; N. Kirkland

Tripler Army Medical Center, Honolulu, HI: LTC (Ret.) C. Mador; C. Uyehara

Uniformed Services University of the Health Sciences, Bethesda, MD: COL (Ret.) K. Chung; COL P. Hickey; LTC J. Livezey; A. Malloy; E. Parmelee;

United States Air Force School of Aerospace Medicine, Dayton, OH: TSgt T. Chao; R. Chapleau; C. Harrington; S. Huntsberger; E. Macias; J. Meyer; C. Starr

United States Coast Guard, Washington, DC: CAPT J. Iskander

Womack Army Medical Center, Fort Bragg, NC: B. Barton; LTC D. Hostler; MAJ K. Lago; C. Maldonado

William Beaumont Army Medical Center, El Paso, TX: M. Wayman

Disclaimer: The authors declare no conflict of interest. Some of the authors are service members or employees of the U.S. Government. This work was prepared as part of their official duties. The contents of this publication are the sole responsibility of the author(s) and do not necessarily reflect the views, opinions, or policies of Uniformed Services University of the Health Sciences (USUHS); the Department of Defense (DoD); the Defense Health Agency (DHA); the Departments of the Army, Navy, or Air Force; Brooke Army Medical Center; Walter Reed National Military Medical Center; Naval Medical Center San Diego; Madigan Army Medical Center; United States Air Force School of Aerospace Medicine; Naval Medical Center.
Portsmouth; Tripler Army Medical Center; Fort Belvoir Community Hospital; or the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. Mention of trade names, commercial products, or organizations does not imply endorsement by the U.S. Government. The investigators have adhered to the policies for protection of human subjects as prescribed in 45 CFR 46.

FINANCIAL SUPPORT

This work was supported by awards from the Defense Health Program (HU00012020067) and the National Institute of Allergy and Infectious Disease (HU00011920111). The protocol was executed by the Infectious Disease Clinical Research Program (IDCRP), a Department of Defense (DoD) program executed by the Uniformed Services University of the Health Sciences (USUHS) through a cooperative agreement by the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. (HJF). This project has been funded in part by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health, under an interagency agreement (Y1-AI-5072). C.B. reports the following support for this work: Department of Defense salary. R.M.M. reports support for this work from 2021-2022.

CONFLICT OF INTEREST

Potential conflicts of interest. S. D. P., T. H. B, D.T. and M.P.S. report that the Uniformed Services University (USU) Infectious Diseases Clinical Research Program (IDCRP), a US Department of Defense institution, and the Henry M. Jackson Foundation (HJF) were funded under a Cooperative Research and Development Agreement to conduct an unrelated phase III COVID-19 monoclonal antibody immunoprophylaxis trial sponsored by AstraZeneca. The HJF, in support of the USU IDCRP, was funded by the Department of Defense Joint Program Executive Office for Chemical, Biological, Radiological, and Nuclear Defense to augment the
conduct of an unrelated phase III vaccine trial sponsored by AstraZeneca. Both of these trials
were part of the US Government COVID-19 response. Neither is related to the work presented
here. C.M.B. reports a leadership or fiduciary role on the IDSA Clinical Affairs Committee.
R.C.M. reports grants or contracts to his institution and unrelated to this work from AiCuris,
Sound Pharmaceutical, and AstraZeneca; consulting fees and honorarium for advisory panel
membership from the Society of Critical Care Medicine; honorarium for lecture from the
California Thoracic Society; travel support from American Thoracic Society, American College
of Chest Physicians, and Society of Critical Care Medicine; U.S. patent for investigational
dengue vaccine candidate (no payments made or current commercial development planned);
DSMB membership (funds to author) for Trauma Insights, LLC; member of SCCM Congress
Program Committee (travel support for official meetings (pre-3/2020)), chair of CHEST
COVID-19 Task Force and Disaster/Global Health Section (travel support for official meetings)
and member of CHEST Scientific Program Committee (travel support for official meetings).
REFERENCES

1. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. The New England journal of medicine 2020; 383(20): 1920-31.
2. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. The New England journal of medicine 2020; 383(27): 2603-15.
3. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. The New England journal of medicine 2021; 384(5): 403-16.
4. Sadoff J, Gray G, Vandebosch A, et al. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. The New England journal of medicine 2021; 384(23): 2187-201.
5. Amit S, Regev-Yochay G, Afek A, Kreiss Y, Leshem E. Early rate reductions of SARS-CoV-2 infection and COVID-19 in BNT162b2 vaccine recipients. Lancet (London, England) 2021; 397(10277): 875-7.
6. Haas EJ, Angulo FJ, McLaughlin JM, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. Lancet (London, England) 2021; 397(10287): 1819-29.
7. Bubar KM, Reinholt K, Kissler SM, et al. Model-informed COVID-19 vaccine prioritization strategies by age and serostatus. Science (New York, NY) 2021; 371(6532): 916-21.
8. Livingston EH, Malani PN, Creech CB. The Johnson &amp; Johnson Vaccine for COVID-19. Jama 2021; 325(15): 1575-.
9. Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. The New England journal of medicine 2021; 385(7): 585-94.
10. Nanduri S, Pilishvili T, Derado G, et al. Effectiveness of Pfizer-BioNTech and Moderna Vaccines in Preventing SARS-CoV-2 Infection Among Nursing Home Residents Before and During Widespread Circulation of the SARS-CoV-2 B.1.617.2 (Delta) Variant - National Healthcare Safety Network, March 1-August 1, 2021. MMWR Morbidity and mortality weekly report 2021; 70(34): 1163-6.
11. Anichini G, Terrosi C, Gandolfo C, et al. SARS-CoV-2 Antibody Response in Persons with Past Natural Infection. The New England journal of medicine 2021; 385(1): 90-2.
12. National Center for I, Respiratory Diseases DoVD. Science Brief: SARS-CoV-2 Infection-induced and Vaccine-induced Immunity. CDC COVID-19 Science Briefs. Atlanta (GA): Centers for Disease Control and Prevention (US), 2020.
13. Gilbert PB, Montefiori DC, McDermott AB, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. Science (New York, NY) 2022; 375(6576): 43-50.
14. Epsi NJ, Richard SA, Laing ED, et al. Clinical, immunological and virological SARS-CoV-2 phenotypes in obese and non-obese military health system beneficiaries. The Journal of Infectious Diseases 2021.

15. Laing ED, Sterling SL, Richard SA, et al. Antigen-based multiplex strategies to discriminate SARS-CoV-2 natural and vaccine induced immunity from seasonal human coronavirus humoral responses. medRxiv : the preprint server for health sciences 2021.

16. Clifton GT, Patti R, Krammer F, et al. SARS-CoV-2 Infection Risk Among Active Duty Military Members Deployed to a Field Hospital - New York City, April 2020. MMWR Morbidity and mortality weekly report 2021; 70(9): 308-11.

17. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. Journal of chronic diseases 1987; 40(5): 373-83.

18. Team TRDC. R: A language environment for statistical computing. R Foundation for Statistical Computing. 2020.

19. Schmidt F, Weisblum Y, Rutkowska M, et al. High genetic barrier to SARS-CoV-2 polyclonal neutralizing antibody escape. Nature 2021; 600(7889): 512-6.

20. Gobbi F, Buonfrate D, Moro L, et al. Antibody Response to the BNT162b2 mRNA COVID-19 Vaccine in Subjects with Prior SARS-CoV-2 Infection. Viruses 2021; 13(3).

21. Wang Z, Schmidt F, Weisblum Y, et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature 2021; 592(7855): 616-22.

22. Saadat S, Rikhtegaran Tehrani Z, Logue J, et al. Binding and Neutralization Antibody Titers After a Single Vaccine Dose in Health Care Workers Previously Infected With SARS-CoV-2. Jama 2021; 325(14): 1467-9.

23. Krammer F, Srivastava K, Alshammary H, et al. Antibody Responses in Seropositive Persons after a Single Dose of SARS-CoV-2 mRNA Vaccine. The New England journal of medicine 2021; 384(14): 1372-4.

24. Bradley T, Grundberg E, Selvarangan R, et al. Antibody Responses after a Single Dose of SARS-CoV-2 mRNA Vaccine. The New England journal of medicine 2021; 384(20): 1959-61.

25. Krammer F. SARS-CoV-2 vaccines in development. Nature 2020; 586(7830): 516-27.

26. Siggins MK, Thwaites RS, Openshaw PJM. Durability of Immunity to SARS-CoV-2 and Other Respiratory Viruses. Trends in Microbiology 2021; 29(7): 648-62.

27. Gazit S, Shlezinger R, Perez G, et al. Comparing SARS-CoV-2 natural immunity to vaccine-induced immunity: reinfections versus breakthrough infections. 2021: 2021.08.24.21262415.

28. Cavanaugh AM, Spicer KB, Thoroughman D, Glick C, Winter K. Reduced Risk of Reinfection with SARS-CoV-2 After COVID-19 Vaccination - Kentucky, May-June 2021. MMWR Morbidity and mortality weekly report 2021; 70(32): 1081-3.

29. Cerqueira-Silva T, Andrews JR, Boaventura VS, et al. Effectiveness of CoronaVac, ChAdOx1 nCoV-19, BNT162b2, and Ad26.COV2.S among individuals with previous
SARS-CoV-2 infection in Brazil: a test-negative, case-control study. The Lancet Infectious Diseases.

30. Nordström P, Ballin M, Nordström A. Risk of SARS-CoV-2 reinfection and COVID-19 hospitalisation in individuals with natural and hybrid immunity: a retrospective, total population cohort study in Sweden. The Lancet Infectious Diseases.

31. Hall V, Foulkes S, Insalata F, et al. Protection against SARS-CoV-2 after Covid-19 Vaccination and Previous Infection. 2022; 386(13): 1207-20.

32. Sidik SM. COVID vaccine plus infection can lead to months of immunity. Nature 2022.

33. Steensels D, Pierlet N, Penders J, Mesotten D, Heylen L. Comparison of SARS-CoV-2 Antibody Response Following Vaccination With BNT162b2 and mRNA-1273. Jama 2021; 326(15): 1533-5.

34. Self WH TM, Rhoads JP, et al. Comparative Effectiveness of Moderna, Pfizer-BioNTech, and Janssen (Johnson & Johnson) Vaccines in Preventing COVID-19 Hospitalizations Among Adults Without Immunocompromising Conditions — United States, March–August 2021. MMWR Morb Mortal Wkly Rep 2021 70:1337–1343.

35. Chung H, He S, Nasreen S, et al. Effectiveness of BNT162b2 and mRNA-1273 covid-19 vaccines against symptomatic SARS-CoV-2 infection and severe covid-19 outcomes in Ontario, Canada: test negative design study. BMJ (Clinical research ed) 2021; 374: n1943.

36. Krause PR, Fleming TR, Peto R, et al. Considerations in boosting COVID-19 vaccine immune responses. Lancet (London, England) 2021.

37. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nature Medicine 2021; 27(7): 1205-11.

38. Rydyznski Moderbacher C, Ramirez SI, Dan JM, et al. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. Cell 2020; 183(4): 996-1012.e19.

39. Naranbhai V, Nathan A, Kaseke C, et al. T cell reactivity to the SARS-CoV-2 Omicron variant is preserved in most but not all individuals. Cell 2022; 185(6): 1041-51.e6.
### Tables:

**Table 1. Clinical and demographic characteristics of 2660 military health system beneficiaries by SARS-CoV-2 infection and/or vaccination history**

| Vaccine                  | SARS-CoV-2 Infection-alone (N=705) | Vaccination-alone (N=932) | Vaccine-after-infection (N=869) | Vaccine-breakthrough-infection (N=154) | Total (N=2660) | *P* value*<sup>a</sup> |
|--------------------------|-------------------------------------|---------------------------|-------------------------------|---------------------------------------|----------------|------------------------|
| Pfizer/BioNTech BNT162b2 | -                                   | 727 (78.0%)               | 701 (80.7%)                   | 134 (87.0%)                           | 1562 (79.9%)  | <0.01                  |
| Moderna mRNA-1273        | -                                   | 200 (21.5%)               | 150 (17.3%)                   | 19 (12.3%)                            | 369 (18.9%)   |                       |
| Janssen/ JNJ-78436735    | -                                   | 5 (0.5%)                  | 18 (2.1%)                     | 1 (0.6%)                              | 24 (1.2%)     |                       |

| **Sex**                  |                                     |                           |                               |                                       |                | 0.81                   |
|--------------------------|-------------------------------------|---------------------------|-------------------------------|---------------------------------------|----------------|------------------------|
| Female                   | 284 (40.3%)                         | 390 (41.8%)               | 347 (39.9%)                   | 60 (39.0%)                            | 1081 (40.6%)  |                       |
| Male                     | 421 (59.7%)                         | 542 (58.2%)               | 522 (60.1%)                   | 94 (61.0%)                            | 1579 (59.4%)  |                       |

| **Age group**            |                                     |                           |                               |                                       |                | < 0.01                |
|--------------------------|-------------------------------------|---------------------------|-------------------------------|---------------------------------------|----------------|------------------------|
| 18-44                    | 448 (63.5%)                         | 726 (77.9%)               | 492 (56.6%)                   | 94 (61.0%)                            | 1760 (66.2%)  |                       |
| 45-64                    | 203 (28.8%)                         | 169 (18.1%)               | 283 (32.6%)                   | 41 (26.6%)                            | 696 (26.2%)   |                       |
| ≥65                      | 54 (7.7%)                           | 37 (4.0%)                 | 94 (10.8%)                    | 19 (12.3%)                            | 204 (7.7%)    |                       |

| **Race**                 |                                     |                           |                               |                                       |                | < 0.01                |
|--------------------------|-------------------------------------|---------------------------|-------------------------------|---------------------------------------|----------------|------------------------|
| White                    | 314 (44.5%)                         | 593 (63.6%)               | 468 (53.9%)                   | 90 (58.4%)                            | 1465 (55.1%)  |                       |
| Hispanic or Latino       | 200 (28.4%)                         | 125 (13.4%)               | 192 (22.1%)                   | 30 (19.5%)                            | 547 (20.6%)   |                       |
| Black                    | 102 (14.5%)                         | 64 (6.9%)                 | 109 (12.5%)                   | 19 (12.3%)                            | 294 (11.1%)   |                       |
| Asian                    | 21 (3.0%)                           | 49 (5.3%)                 | 28 (3.2%)                     | 3 (1.9%)                              | 101 (3.8%)    |                       |
| Others                   | 68 (9.6%)                           | 101 (10.8%)               | 72 (8.3%)                     | 12 (7.8%)                             | 253 (9.5%)    |                       |

| **CCI<sup>b</sup>**      |                                     |                           |                               |                                       |                | < 0.01                |
|--------------------------|-------------------------------------|---------------------------|-------------------------------|---------------------------------------|----------------|------------------------|
| >5                       | 23 (3.3%)                           | 3 (0.3%)                  | 34 (3.9%)                     | 4 (2.6%)                              | 64 (2.4%)     |                       |
| 3-4                      | 28 (4.0%)                           | 11 (1.2%)                 | 44 (5.1%)                     | 5 (3.2%)                              | 88 (3.3%)     |                       |
| 1-2                      | 124 (17.6%)                         | 37 (4.0%)                 | 180 (20.7%)                   | 17 (11.0%)                            | 358 (13.5%)   |                       |
| 0                        | 530 (75.2%)                         | 881 (94.5%)               | 611 (70.3%)                   | 128 (83.1%)                           | 2150 (80.8%)  |                       |

| **Severity**             |                                     |                           |                               |                                       |                | < 0.01                |
|--------------------------|-------------------------------------|---------------------------|-------------------------------|---------------------------------------|----------------|------------------------|
| Inpatient                | 168 (23.8%)                         | -                         | 142 (16.3%)                   | 9 (5.8%)                              | 319 (18.5%)   |                       |
| Outpatient               | 537 (76.2%)                         | -                         | 727 (83.7%)                   | 145 (94.2%)                           | 1409 (81.5%)  |                       |

| **Sampling time<sup>c</sup>** |                                     |                           |                               |                                       |                | < 0.01                |
|-------------------------------|-------------------------------------|---------------------------|-------------------------------|---------------------------------------|----------------|------------------------|
| Time since infection          | 32 (20)                             |                           |                               |                                       |                |                       |
| Time since latest antigenic exposure<sup>d</sup> | 76 (79)                             | 78 (70)                   | 89 (63)                       |                                       |                |                       |

*<sup>a</sup>*n x k Fisher's exact test  
*<sup>b</sup>*CCI = Charlson Comorbidity Index  
*<sup>c</sup>*Median (Interquartile Range).  
*<sup>d</sup>*Time since final dose of vaccine or infection, whatever is latest  
Vaccination status determined through Military Health System Data Repository (MDR) record, case report form (CRF), and/or self-report questionnaire.
Table 2. Univariable and multivariable models to compare adjusted anti-spike log_{10} MFI response in different categories of infection and vaccination

| Category                          | Coefficient (95% CI) | P value | Adjusted Coefficient\(^a\) (95% CI) | P value |
|----------------------------------|----------------------|---------|-------------------------------------|---------|
| Age group: 45-64                 | 0.03 (0.02 to 0.05)  | <0.01   | 0.02 (0.01 to 0.04)                 | <0.01   |
| Age group: ≥65                   | 0.06 (0.04 to 0.08)  | <0.01   | 0.03 (0.01 to 0.05)                 | 0.01    |
| Sex: Male                        | <0.01 (-0.01 to 0.01)| 0.81    | <0.01 (-0.01 to 0.01)               | 0.5     |
| CCI index: ≥5                    | 0.04 (0 to 0.08)     | 0.04    | 0.01 (-0.04 to 0.03)                | 0.91    |
| CCI index: 3-4                   | 0.05 (0.01 to 0.08)  | 0.01    | 0.02 (-0.01 to 0.05)                | 0.25    |
| CCI index: 1-2                   | 0.05 (0.03 to 0.07)  | <0.01   | 0.03 (0.01 to 0.04)                 | <0.01   |
| Group: Vaccination-after-infection\(^b\) | 0.1 (0.08 to 0.11)  | <0.01   | 0.13 (0.12 to 0.15)                 | <0.01   |
| Group: Vaccine-breakthrough-infection\(^b\) | 0.13 (0.1 to 0.15) | <0.01   | 0.18 (0.16 to 0.21)                 | <0.01   |
| Group: Vaccination-alone\(^b\)   | 0.03 (0.01 to 0.04)  | <0.01   | 0.07 (0.06 to 0.09)                 | <0.01   |
| Sampling time                    | <0.01 (<0.01 to <0.01)| < 0.01 | <0.01 (<0.01 to <0.01)              | < 0.01  |

Number of observations 2254

\(^a\)Adjusted for age group, sex, CCI, Group, Sampling time

\(^b\)Ref: SARS-CoV-2 infection-alone

CCI = Charlson Comorbidity Index

MFI = median fluorescence intensity

Sampling time refers to last antigenic exposure, i.e., time since final dose of vaccine or infection, whatever is latest

Log_{10} MFI coefficients are exponentiated in the text for interpretability
Table 3: Univariable and multivariable models to identify correlates of peak post-vaccine anti-spike log$_{10}$ IgG among those vaccinated with and without history of infection

|                        | Coefficient (95% CI) | $P$ value | Adjusted Coefficient$^a$ (95% CI) | $P$ value |
|------------------------|----------------------|-----------|-----------------------------------|-----------|
| Age group: 45-64       | $<0.01$ (-0.01 to 0.02) | 0.65      | -0.01 (-0.02 to 0)                | 0.09      |
| Age group: ≥65         | 0.03 (0.01 to 0.05)   | 0.01      | -0.01 (-0.02 to 0.02)             | 0.89      |
| Sex: Male              | $<0.01$ (-0.01 to 0.01) | 0.8       | -0.01 (-0.01 to 0.01)             | 0.49      |
| CCI: ≥5                | 0.04 (0 to 0.08)      | 0.04      | -0.01 (-0.05 to 0.03)             | 0.66      |
| CCI: 3-4               | 0.06 (0.02 to 0.09)   | $<0.01$   | 0.03 (0 to 0.06)                  | 0.04      |
| CCI: 1-2               | 0.04 (0.02 to 0.06)   | $<0.01$   | 0.01 (-0.01 to 0.03)              | 0.26      |
| Sampling time          | $<0.01$ (-0.01 to -0.01) | $<0.01$  | $<0.01$ (-0.01 to -0.01)          | $<0.01$  |
| Vaccine Product: Pfizer/BioNTech BNT162b2$^b$ | 0.06 (0.01 to 0.11)   | 0.01      | 0.09 (0.05 to 0.13)               | $<0.01$  |
| Vaccine Product: Moderna/mRNA-1273$^b$ | 0.08 (0.03 to 0.12)   | $<0.01$   | 0.11 (0.07 to 0.16)               | $<0.01$  |
| History of SARS-CoV-2 Infection History$^c$ | 0.07 (0.06 to 0.08)   | $<0.01$   | 0.08 (0.06 to 0.09)               | $<0.01$  |

Number of observations: 1395

$^a$Adjusted for age group, sex, CCI, sampling time, vaccine product, and prior SARS-CoV-2 infection

$^b$Ref: Janssen/ JNJ-78436735

$^c$Ref: No history of SARS-CoV-2 infection

CCI = Charlson Comorbidity Index

Sampling time refers to last antigenic exposure, i.e., time since final dose of vaccine or infection, whatever is latest
Table 4. Univariable and multivariable models to identify correlates of peak anti-spike log\(_{10}\) MFI response in those vaccinated with prior infection history

|                           | Coefficient (95% CI)         | P value | Adjusted Coefficient\(^a\) (95% CI) | P value |
|---------------------------|------------------------------|---------|------------------------------------|---------|
| Age group: 45-64          | <0.01 (-0.01 to 0.01)        | 0.66    | <0.01 (-0.01 to 0.01)              | 0.72    |
| Age group: 65+            | 0.01 (<0.01 to 0.03)         | 0.18    | 0.01 (-0.01 to 0.02)               | 0.52    |
| Sex: Male                 | 0.01 (<0.01 to 0.01)         | 0.3     | <0.01 (-0.01 to 0.01)              | 0.41    |
| CCI: ≥5                   | 0.01 (-0.01 to 0.04)         | 0.33    | <0.01 (-0.02 to 0.03)              | 0.86    |
| CCI: 3-4                  | 0.02 (<0.01 to 0.04)         | 0.03    | 0.02 (0 to 0.04)                   | 0.06    |
| CCI: 1-2                  | 0.01 (-0.01 to 0.02)         | 0.38    | <0.01 (-0.01 to 0.02)              | 0.67    |
| Severity of prior infection - Inpatient\(^b\) | <0.01 (-0.01 to 0.02)        | 0.46    | <0.01 (-0.01 to 0.01)              | 0.88    |
| Sampling time\(^c\)       | <-0.01 (-<0.01 to <-0.01)    | <0.01   | <-0.01 (-<0.01 to <-0.01)          | <0.01   |

Number of observations 653

\(^a\)Adjusted for age, sex, CCI, severity, sampling time

\(^b\)Ref: Outpatient

\(^c\)Sampling time refers to time since final dose of vaccination

Vaccination refers to mRNA vaccine

CCI = Charlson Comorbidity Index
Table 5: Longitudinal linear mixed modeling to estimate decay kinetics of log_{10} anti-spike IgG response to antigenic exposure (N = 1160).

| Coefficient                    | P value  |
|--------------------------------|----------|
| Sampling Time                  | -<0.01 (-<0.01 to -<0.01) | <0.01 |
| Vaccination-after-infection    | 0.13 (0.09 to 0.16)       | <0.01 |
| Vaccine-breakthrough-infection | 0.1 (0.06 to 0.14)        | <0.01 |
| Vaccination-alone              | 0.15 (0.09 to 0.21)       | <0.01 |
| Sampling time*Group: Vaccine-after-infection | <0.01 (<0.01 to <0.01) | 0.03 |
| Sampling time*Group: Vaccine-breakthrough-infection | <0.01 (<0.01 to <0.01) | <0.01 |
| Sampling time*Group: Vaccination-alone | -<0.01 (-<0.01 to -<0.01) | 0.02 |

Number of observations 1160

*Ref: SARS-CoV-2 infection-alone
Sampling time refers to last antigenic exposure, i.e., time since final dose of vaccine or infection, whatever is latest
Model fit to all longitudinal data and including group, sampling time, and an interaction term of sampling time and group
The asterisk (*) is used to indicate interactions among the variables that it joins
FIGURE LEGENDS

**Figure 1**: Peak anti-spike-IgG MFI by sampling time (sampling time defined as time since vaccination or infection, whatever is latest). Y-axis depicts anti-spike-IgG MFI values and X-axis depicts sampling time. Each data point represents a single subject with a single peak humoral response. LOESS curves were fit to (A) SARS-CoV-2 infection-alone (those who tested SARS-CoV-2 positive and did not receive any subsequent vaccination), (B) vaccination-alone without a known history of SARS-CoV-2 infection, (C) vaccine-after-infection (those who tested SARS-CoV-2 positive and then received a complete series of vaccination), and (D) vaccine-breakthrough-infection (those who were infected by SARS-CoV-2 after complete doses of vaccination). These curves report moving averages but are not adjusted rates of decay; 95% CI are shaded in pink. Orange dots depict pre-vaccination samples and green data points depict sampling greater than two weeks after complete vaccination.

**Figure 2**: Unadjusted comparison of peak observed anti-spike-IgG median fluorescence intensity (MFI) by category of infection and/or vaccination. P value determined by Mann Whitney U test. These comparisons do not adjust for sampling time which varies by group. Boxplots denote median, first quartile (25th percentile) and third quartile (75th percentile) of anti-spike-IgG MFI levels (Y-axis) and each group (X-axis), representing SARS-CoV-2 infection-alone (yellow), vaccination-alone (blue), vaccine-after-infection (light green) and vaccine-breakthrough-infection (coral). SARS-CoV-2 infection-alone and vaccination-alone did not portray any statistically significant difference, but vaccination-after-infection vaccine-breakthrough SARS-CoV-2 infection shows greater humoral response compared to infection-alone or vaccination-alone.

**Figure 3**: Anti-spike-IgG MFI by sampling time (time since vaccination or infection, whatever is latest), restricted to longitudinal analysis using at least two sera samples per subject, stratified by SARS-CoV-2 infection-alone (yellow), vaccination-alone (blue), vaccine-after-infection (light green), and vaccine-breakthrough-infection (coral). Y-axis depicts anti-spike-IgG MFI values and X-axis depicts sampling time. Each data point represents a sera sample. The solid line is the estimated slope derived from mixed-effects regression models in Table 5; shaded area depicts confidence interval.
Figure 1

A. SARS-CoV-2 infection-alone

B. Vaccination-alone

C. Vaccine-after-infection

D. Vaccine-breakthrough-infection

Figure 2

229x79 mm (.80 x DPI)
Figure 3

SARS-CoV-2 Infection-alone
Vaccination-alone
Vaccine-after-infection
Vaccine-breakthrough-infection

229x159 mm (.80 x DPI)