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Determinants of the Longitudinal Antibody Response Following Initial mRNA Vaccination

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ABSTRACT (250-300 words)

Objectives. We sought to understand the demographic and clinical factors associated with variations in longitudinal antibody response following completion of 2-dose regiment of BNT162b2 vaccination.

Design. This study is a 10-month longitudinal cohort study of healthcare workers and serially measured anti-spike protein IgG (IgG-S) antibody levels, using mixed linear models to examine their associations with participant characteristics.

Setting. Large multi-site academic medical center in Southern California.

Participants. A total of 828 healthcare workers met inclusion criteria including completion of an initial two-dose course of BNT162b2 vaccination, complete clinical history and at least 2 blood samples for analysis. Patients had an average age of 45±13 years, were 70% female, and 7% with prior SARS-CoV-2 infection.

Results. Vaccine induced IgG-S levels remained in the positive range for 99·6% of individuals up to 10 months after initial 2-dose vaccination. Prior SARS-CoV-2 infection was the primary correlate of sustained higher post-vaccination IgG-S levels (partial-r2=0·133), with a 1·74±0.11 SD higher IgG-S response (P<0·001). Female sex (P<0·001), younger age (P<0·001), and absence of hypertension (P=0·041) were also associated with persistently higher IgG-S responses. Notably, prior SARS-CoV-2 infection augmented the associations of sex (interaction P=0·033) and modified the associations of hypertension (interaction P=0·006), such that infection-naïve individuals with hypertension had persistently lower IgG-S levels (P=0·005) whereas prior-infected individuals with hypertension exhibited higher IgG-S levels (P=0·06) that remained augmented over time.

Conclusions. While the IgG-S antibody response remains in the positive range for up to 10 months following initial mRNA vaccination in most adults, determinants of sustained higher antibody levels include prior SARS-CoV-2 infection, female sex, younger age, and absence of

hypertension. Certain determinants of the longitudinal antibody response appear significantly modified by prior infection status. These findings offer insights regarding factors that may influence the 'hybrid' immunity conferred by natural infection combined with vaccination.

Keywords

SARS-CoV-2, longitudinal antibody response, sex differences, hypertension

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Evaluation of demographic and clinical characteristics associated with variable longitudinal antibody response following BNT162b2 vaccination.
- Among the longest follow up studies of COVID-19 vaccine associated humoral immune response
- Large, diverse study cohort
- Prospective study design
- Assessment of humoral, but not T-cell mediated antibody response

INTRODUCTION

Exposure to SARS-CoV-2 or its subunits, via natural infection or vaccination, can elicit a humoral immune response that is measurable in the circulation and correlated with relative protection from future infectious disease.[1-4] Recent studies have indicated that this quantifiable humoral response wanes over time – as soon as 3 to 6 months following either natural infection or initial administration of a SARS-CoV-2 vaccination.[5-7] While certain population subsets may experience more or less durable immunity from an initial natural or vaccine exposure, the demographic and clinical characteristics that may influence temporal variations in provoked humoral immune response currently remain unclear.[8]

Given lack of clarity regarding the factors that could promote accelerated versus delayed decline in acquired SARS-CoV-2 immunity, along with concern for immunocompromised persons at the highest risk for opportunistic infections, governments worldwide have made provisions to offer additional 'booster' vaccine doses.[9-11] Amidst rollout of the booster vaccinations, there remains equipoise regarding their appropriateness for individuals suspected of having more robust immunity following initial vaccination – including those recovered from prior SARS-CoV-2 infection and younger healthy persons. In fact, emerging data suggest that individuals who have been both fully vaccinated and previously infected with SARS-CoV-2 are likely to benefit from a 'hybrid immunity' that offers durable protection from infection in terms of both strength and longevity.[12-15]

To improve our understanding of the longitudinal immune response following initial SARS-CoV-2 vaccination – and the factors associated with variations in this response – we examined the demographic and clinical correlates of anti-spike IgG antibody (IgG-S) levels measured serially in a large cohort of fully vaccinated adults.

METHODS

Study Sample

We conducted serial serological assays a longitudinal cohort study of healthcare workers who received vaccination with Pfizer-BioNTech (BNT162b2) at our medical center in Southern California, with study design and sampling procedures detailed previously.[16] Briefly, participants completed surveys on medical history, exposures, and symptoms at baseline and at serial timepoints over the course of the study. History of SARS-CoV-2 infection prior to vaccination was determined based on self-report along with adjudication of medical records and confirmed presence of antibodies targeting the viral nucleocapsid protein [IgG(N)]. Of the total 1,703 healthcare worker participants in the source cohort, we excluded individuals from the current analysis if they did not receive the BNT162b2 vaccine (N=23), their medical history could not be confirmed (N=14), they developed a breakthrough infection any time after first vaccine dose (N=27), or they did not provide at least 2 blood samples for serology following completion of their second vaccine dose (N=796), resulting in a final cohort of 828 individuals (**Supplemental Figure 1** and **Supplemental Table 1**). All participants provided written informed consent for all protocols, which were reviewed and approved by the Cedars-Sinai institutional review board.

Serology

Serological assays for antibodies to the receptor binding domain (RBD) of the S1 subunit of the viral spike protein [IgG (S-RBD)], and IgG(N) were performed using the Abbott SARS-CoV-2 IgG II assay and SARS-CoV-2 IgG assay, respectively (Abbott Labs, Abbott Park, IL). Antibody levels were measured from plasma samples collected at the following time points: before or up to 3 days after dose 1; within 7 to 21 days after dose 1; within 7 to 21 after dose 2; and then at 8, 16, 24, 32, and 40 weeks after dose 2. We considered an IgG(N) S/C of ≥1·4 as denoting definitive seropositive status due to prior SARS-CoV-2 exposure based on a previously established thresholds.[17]

Statistical Analyses

For descriptive statistics, we used analysis of variance to test for differences between continuous normally distributed variables, Kruskal-Wallis rank sum tests for non-normal continuous variables, and chi squared test for categorical variables. We used mixed-effect linear modeling to estimate the mean and 95% confidence interval of log(10)IgG-S levels in relation to time since the date of complete vaccination (i.e. dose 2), with time expressed using natural cubic splines. For longitudinal modeling, we used the AIC to select the optimal number of knots, which was optimized when using 4 knots placed at the 5th, 35th, 65th, and 95th percentiles. We treated repeated measures for each participant as random effect and additionally adjusted for age, sex, race, ethnicity, obesity, hypertension, and the Charlson comorbidity index[18] calculated based on the combination of information collected from medical history surveys and the electronic health record.[16 19] In secondary analyses, we repeated multivariable-adjusted mixed-effect regression analyses including multiplicative interaction terms for any significantly associated demographic or clinical variables, to assess for potential effect modification of the anticipated relation between prior SARS-CoV-2 infection on longitudinal log(10)IgG-S trajectory. We conducted all statistical analyses using R (v4.1.1) and considered statistical significance as a two-tailed P value less than 0·05.

Patient and Public Involvement

Patients and the public were not involved in the development of this study.

RESULTS

The demographic and clinical characteristics of our study sample are shown in **Supplemental Table 1**. As shown, there were no clinically meaningful differences in age, sex, or clinical comorbidities between individuals with and without prior SARS-CoV-2 infection. All prior infected individuals not only survived the index infection (with only 5% requiring hospitalization), and all were considered to have recovered successfully (without persistent or recurrent symptoms).

In spline analyses of the longitudinal trajectory of response in log(10)IgG-S levels following vaccination, we observed that 99·6% of all healthcare worker participants had repeated values that remained within the positive reference range of ≥ log(10)50 AU/mL over the entire follow-up period of up to 40 weeks (**Figure 1**). In multivariable-adjusted models examining demographic and clinical correlates of longitudinal IgG-S levels, we found that prior SARS-CoV-2 infection was associated with substantially higher antibody levels with prior infected individuals exhibiting an almost 1·7-fold higher standard deviation in log(10)IgG-S levels compared to never infected individuals (**Table 1**). Whereas younger age (below the median cohort age of 42 years) and female sex were also significantly associated with higher IgG-S levels over the duration of the study period (**Table 1**), prior SARS-CoV-2 infection was the predominant determinant with the largest model partial r2 value of 0·134. These results indicate that 13·4% of the observed variation in longitudinal IgG-S levels was attributable to prior infection status even after accounting for other covariates in the model that include age, sex, race, ethnicity, and the Charlson comorbidity burden index.

In secondary analyses, we found that the interaction between age and prior infection status on longitudinal IgG-S levels was non-significant (P=0·45, **Figure 2**) although, in exploratory stratified analyses, older age was significantly associated with lower IgG-S response among infection-naïve individuals whereas no significant age-based association was seen in

prior-infected individuals (**Supplemental Table 3**). Notably, we observed significant interactions of male sex with prior infection (P=0·033) and of hypertension with prior infection (interaction P=0·006). Accordingly, in analyses stratified by prior infection status, male compared to female sex was associated with greater magnitude of difference in IgG-S level in prior infected (beta -0·72 [se 0·33], P=0·032) compared to never infected individuals (beta -0·24 [se 0·06], P=<0·001) (**Supplemental Table 3**); this finding was also demonstrated by longitudinal splines in **Figure 3**. Notably, as shown in **Figure 4**, presence versus absence of hypertension was significantly associated with lower IgG-S level in never infected persons (beta -0·23 [se 0·08], P=0·005) while concurrently related to higher IgG-S levels in prior infected individuals (beta 0·96 [se 0·50], P=0·06) (**Supplemental Table 3**). Analyses stratified by age, sex, and prior infection status demonstrated concordant results (**Supplemental Table 4**).

DISCUSSION

From our study of repeated serological measures performed in a large cohort with two-dose initial BNT162b2 vaccination, there were several key findings. First, we found that the vast majority of adults in our cohort maintained appropriate elevations of IgG-S antibody levels within the positive reference range up to 10 months following initial complete vaccination. Second, the primary differentiator of antibody response trajectory was prior SARS-CoV-2 infection, with a relatively fixed magnitude of variance that lasted throughout the follow up period. Finally, correlates of persistently higher longitudinal antibody response level included female sex, younger age, and absence of hypertension in analyses adjusting for race, ethnicity, and comorbidities. Intriguingly, the longitudinal effect of prior infection status was differentially modified by these associations – particularly sex and hypertension status.

Extending from prior studies,[5 6] we repeated serological measures up to 10 months following initial SARS-CoV-2 vaccination in a large cohort of adults who receive their BNT162b2 vaccinations according to the standardized 2-dose schedule. While observing an initial peak and then steady decline in the absolute levels of IgG-S antibody response, as seen in other studies, we also found a relatively consistent pattern of longitudinal response that almost invariably involved levels remaining in the positive range during the follow-up period. Specifically, we found that the average trajectory of response in IgG-S antibody levels peaks within the first 2 to 8 weeks after the second vaccine dose and then declines towards a relative plateau – seen on the log10 scale – that lasts up to 40 weeks. Notwithstanding continued reductions in the absolute IgG-S antibody levels, the relative plateau on the log scale signals an attenuation in the rate of decline and is consistent with the longitudinal patterns of post-vaccination antibody titer response that has been reported for other viruses (e.g. influenza) and predicted for SARS-CoV-2.[20-22] Although the threshold of 50 AU/mL for absolute IgG-S antibody levels is validated with 99·5% specificity for detecting antibodies specific to the SARS-CoV-2 spike protein, and

the exact quantitative thresholds that may correspond to effective immunity remains unclear, a relative plateau in the log10 scale presence of IgG-S offers some assurance of continued memory B cell activation potentially indicative of an even broader immunological reserve.

In addition to the overall trajectory common to most participants, we found that the primary and persistent differentiator of antibody response trajectory was prior SARS-CoV-2 infection. Extending from prior studies that examined serological responses up to 6 months after SARS-CoV-2 vaccination,[5] we observed a relatively fixed magnitude of difference in provoked IgG-S levels – consistently higher in prior infected compared to never infected individuals – persisting beyond 10 months. The absence of any indication that this difference is narrowing suggests that the 'hybrid' immunity obtained from the combination of natural infection and vaccination is likely to endure over time – a phenomenon consistent with recent findings of dynamic memory B cell activation and clonal turnover in individuals exposed to both natural infection and vaccine.[12] Furthermore, and intriguingly, prior infected individuals had persistently elevated post-vaccine antibody levels that did not differ by age – indicating minimal influence of age-related humoral deficiency on the 'hybrid' or dose-boosted effect.[23 24] By contrast, the previously reported female advantage in antibody response to SARS-CoV-2 vaccination[6 25] appeared accentuated by prior infection such that previously infected females tended to exhibit the most pronounced as well as persistently elevated antibody response. Females are known to generate antibody responses to a variety of viral vaccines that are almost twice as high as the responses seen in males.[26] Augmentation and persistence of this sex difference in the setting of 'hybrid' SARS-CoV-2 exposure points to a female advantage in at least humoral immunity that could represent a mechanistic contributor to the female advantage seen in COVID-19 related outcomes.

Our results regarding the associations of hypertension with longitudinal antibody response are especially notable. Extending from prior studies focused on initial post-vaccine

effects,[27 28] we found that presence of hypertension was associated with an overall lower level antibody level response that was consistent over time and persisted for up to 10 months. Intriguingly, we also found that among persons with prior SARS-CoV-2 infection, the association of hypertension status on longitudinal IgG-S antibody response was reversed. In effect, longitudinal antibody levels are profoundly increased among hypertensive participants with prior COVID-19 compared to without prior COVID-19. Previous studies have demonstrated a more robust antibody response following native infection among hypertensive individuals – attributed to a combination of increased sympathetic drive and an underlying inflammatory state serving to enhance immune activation.[29 30] These same factors have been hypothesized as contributors to the greater mortality risk seen among hypertensive COVID-19 patients. In light of the lower antibody response to vaccination seen in hypertensives overall, the paradoxically higher response seen in hypertensives with prior COVID-19 is similar to the trend seen for older-aged individuals with prior infection. In both situations, a pre-existing relative deficiency in immune reserve is superseded by the effects of having been directly exposed to and then recovered from COVID-19. Importantly, these effects appear to persist in the population over time.

Several limitations of this study merit consideration. First, all participants received the Pfizer-BioNTech (BNT162b2) vaccine, limiting generalizability to other vaccines, although variable waning of antibody levels following other SARS-CoV-2 vaccines has been described.[8] There also exists potential bias in the study population, as not all participants provided longitudinal serology data, although there were negligible clinically meaningful differences between those with and without adequate serology data for inclusion. Importantly, all prior infected individuals in our study were not only survivors of COVID-19 but were predominantly less severely affected with only 5% requiring hospitalizations, all of which lasted less than 5 days, and none reporting continued or recurrent symptoms following recovery from the index infection. This issue is particularly important to consider when interpreting interaction analyses,

as a provoked humoral immune response that is augmented to a level that is sufficient for countering infection is likely different from an exaggeration in response that may contribute to end-organ dysfunction or continued symptoms. Furthermore, the average age of our healthcare worker cohort was relatively younger than that of the general population, even while including a relatively broad range of ages from 19 to 82 years. Finally, this study was not designed to assess the extent to which natural infection or vaccine augmented and sustained antibody levels represent relatively greater immunity against emerging novel SARS-CoV-2 variants. We also do not address non-humoral related immune protection, which may protect or predispose to future infections.

In summary, our findings indicate that completion of a two-dose mRNA vaccine regimen provokes an IgG-S antibody response that is not only enhanced but also persistent among individuals with prior native SARS-CoV-2 infection when compared to those without prior infection. Further, our results demonstrate potential sex and hypertension specific variations in the longitudinal response to single vs dual antigenic exposure that may guide more tailored assessments of individual-level risks for future infection. In particular, the role of hypertension as a potential potent modifier of antibody response, with divergent post-vaccination effects between those with and without prior infection, may reflect key differences in physiologically mediated immune response among those with and without high blood pressure. These findings may allow for allocation of still limited vaccine resources by targeting individuals most likely to benefit from additional vaccine doses.

CONTRIBUTORSHIP STATEMENT

JEE contributed via Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, and Project Administration. SJ contributed via Conceptualization, Resources, Data Curation, and Writing - Review & Editing. YL and MW contributed via Methodology, Formal Analysis, Writing - Original Draft, Writing - Review & Editing, and Visualization. BW, YHK, BK, TW, TTN, and MA contributed via Resources and Data Curation. BC contributed via Formal Analysis, Writing - Review & Editing, and Supervision. PGB, NS, and MD contributed via Validation, Investigation, and Writing - Review & Editing. JCP contributed via Methodology, Investigation, and Writing - Review & Editing. ECF contributed via Investigation and Resources. JLS, HSG, PC, and SCJ, contributed via Investigation, Resources, and Writing - Review & Editing. MJ contributed via Investigation, Resources, Writing - Review & Editing, and Funding Acquisition. SS, JFB, JEVE, MBM, MA, and GYM contributed via Investigation and Writing - Review & Editing. JGB contributed via Investigation, Writing - Review & Editing, Project Administration, and Funding Acquisition. DPBM contributed via Investigation, Writing - Review & Editing, and Project Administration. SC and KS Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project Administration, and Funding Acquisition. The corresponding authors attest that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

CONFLICTS OF INTEREST

JCP, ECF, and JLS work for Abbott Diagnostics, a company that performed the serological assays on the biospecimens that were collected for this study. The remaining authors have no disclosures to report.

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ETHICS APPROVAL

This study was approved by the Cedars-Sinai Institutional Review Board (IRB) (CORALE Study00000621). All participants provided written informed consent for all protocols.

DATA SHARING

Due to the sensitive nature of the data collected for this study, requests to access the dataset from qualified researchers trained in protocols on the protection of human subjects may be sent to Cedars-Sinai Medical Center at biodatacore@cshs.org. The manuscript's guarantors (JEE, SC, KS) affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Table 1. Clinical and demographic correlates of longitudinal anti-spike IgG antibody response following complete initial mRNA vaccination.

	Beta*	SE	P	Partial r2
Prior SARS-CoV-2 infection	1·74	0·11	<0·001	0·134
Age, year	-0·01	0·00	<0·001	0·016
Male sex	-0·27	0·06	<0·001	0·013
Non-white race	-0·00	0·06	0·99	0·000
Hispanic ethnicity	0·02	0·10	0·80	0·000
Obesity	0·03	0·09	0·77	0·000
Hypertension	-0·17	0·08	0·041	0·003
Charlson comorbidity index	-0·02	0·03	0·56	0·000

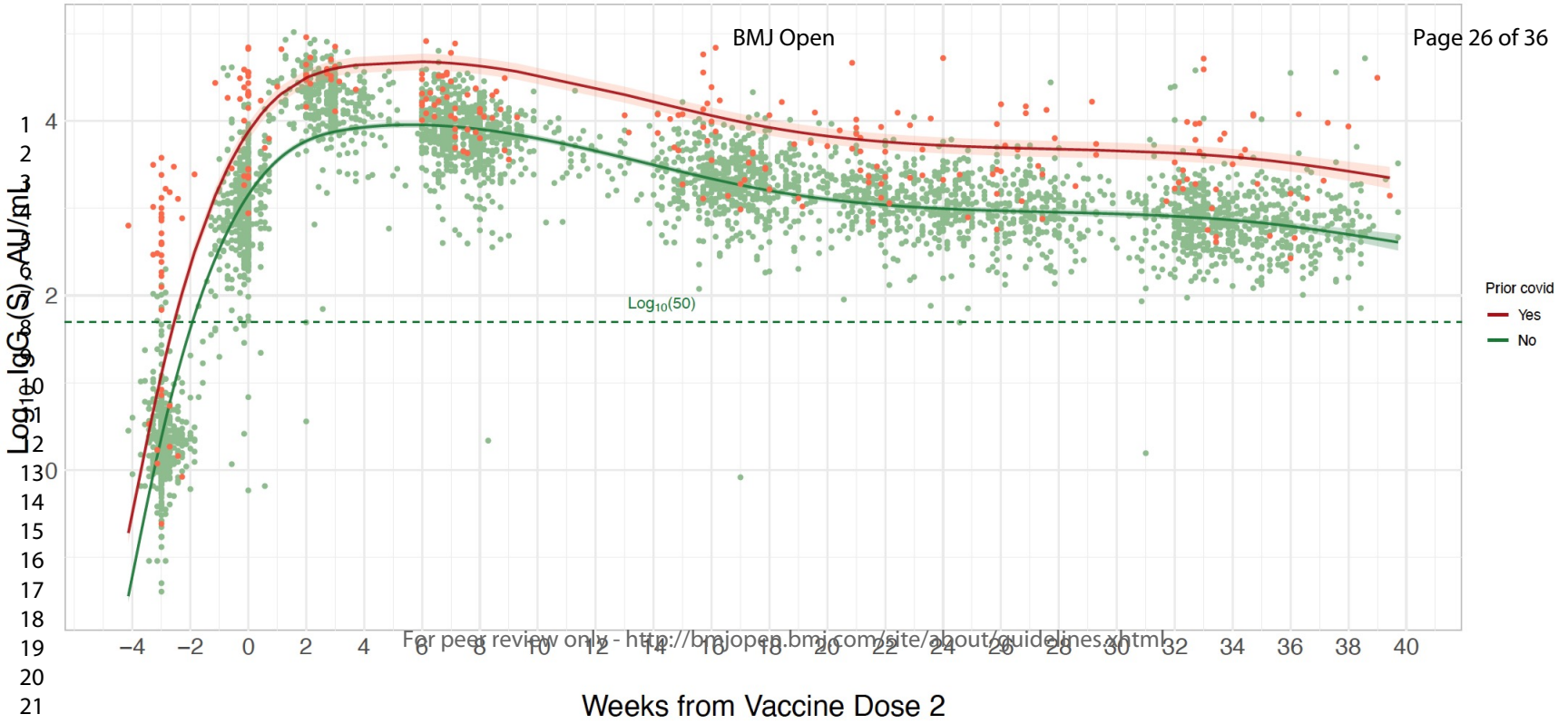
*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable).

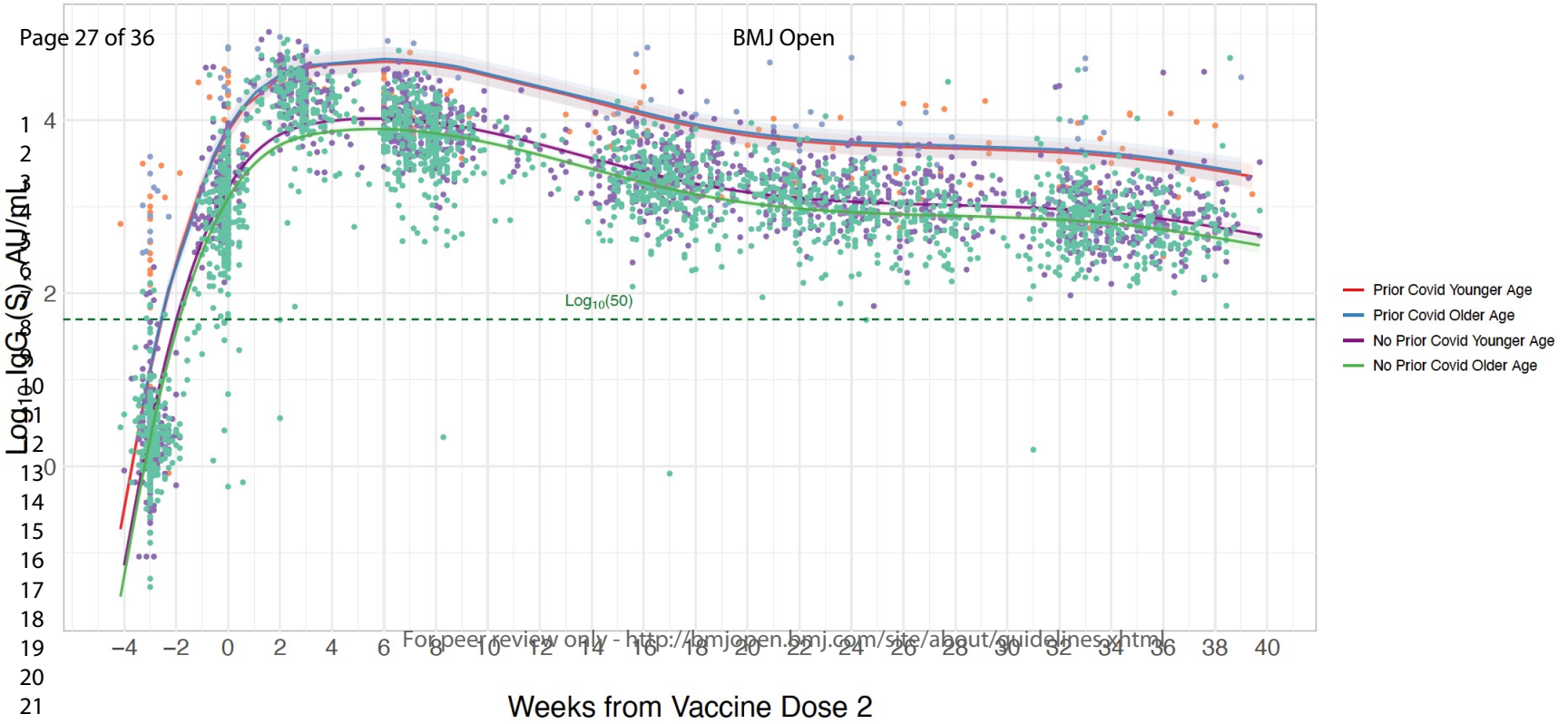
Figure 1. Longitudinal trajectory of IgG-S antibody levels following completed BNT162b2 vaccination. Multivariable-adjusted longitudinal trajectories are shown for individuals with a history of prior COVID-19 infection (orange line) for those without prior COVID-19 infection (green line). Longitudinal estimates with 95% confidence limits (shaded areas) are adjusted for age, sex, and hypertension.

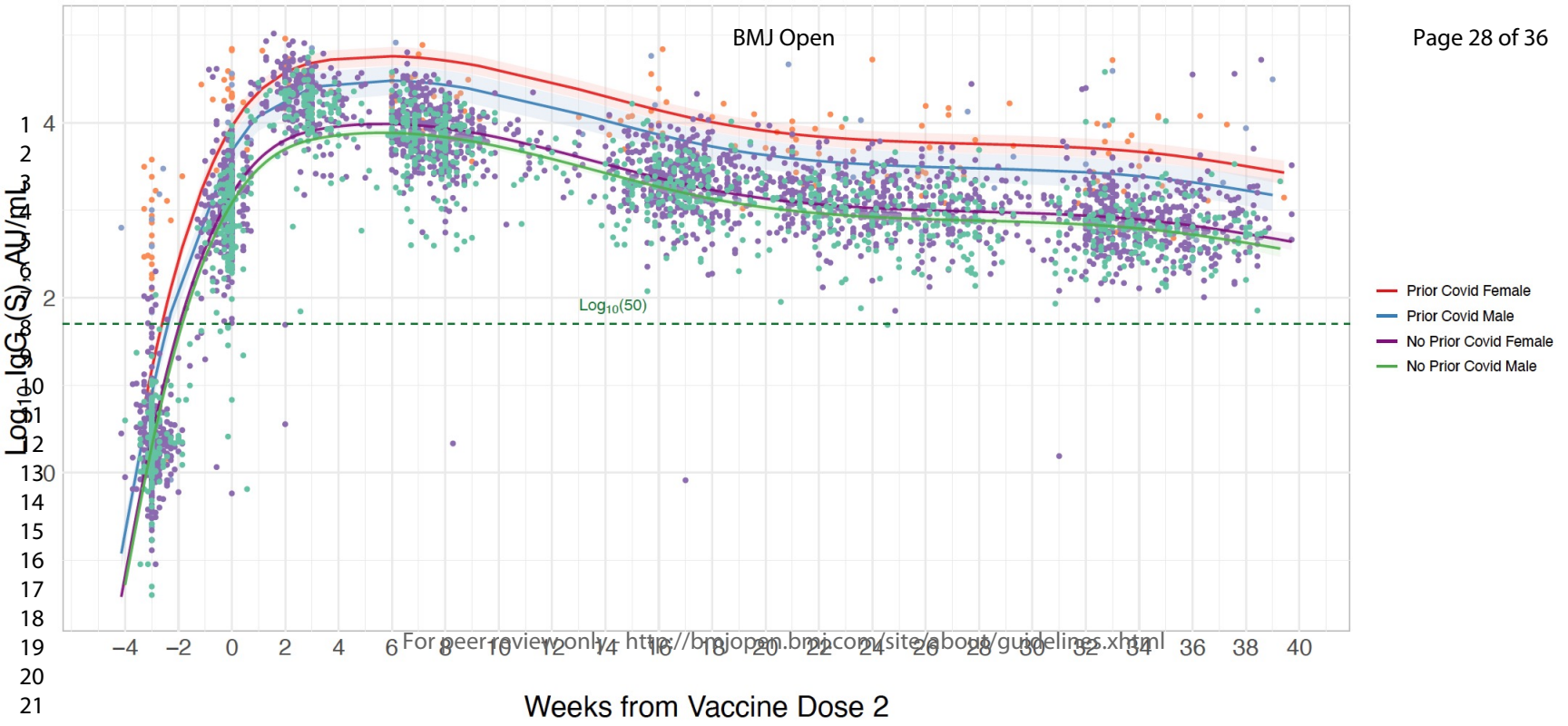
Figure 2. Longitudinal trajectory of IgG-S antibody levels following completed BNT162b2 vaccination by prior infection status and age. Multivariable-adjusted longitudinal trajectories are shown for individuals with a history of prior COVID-19 infection for those without prior COVID-19 infection, including an interaction for age (above vs below median cohort age). Longitudinal estimates with 95% confidence limits (shaded areas) are adjusted for sex and hypertension.

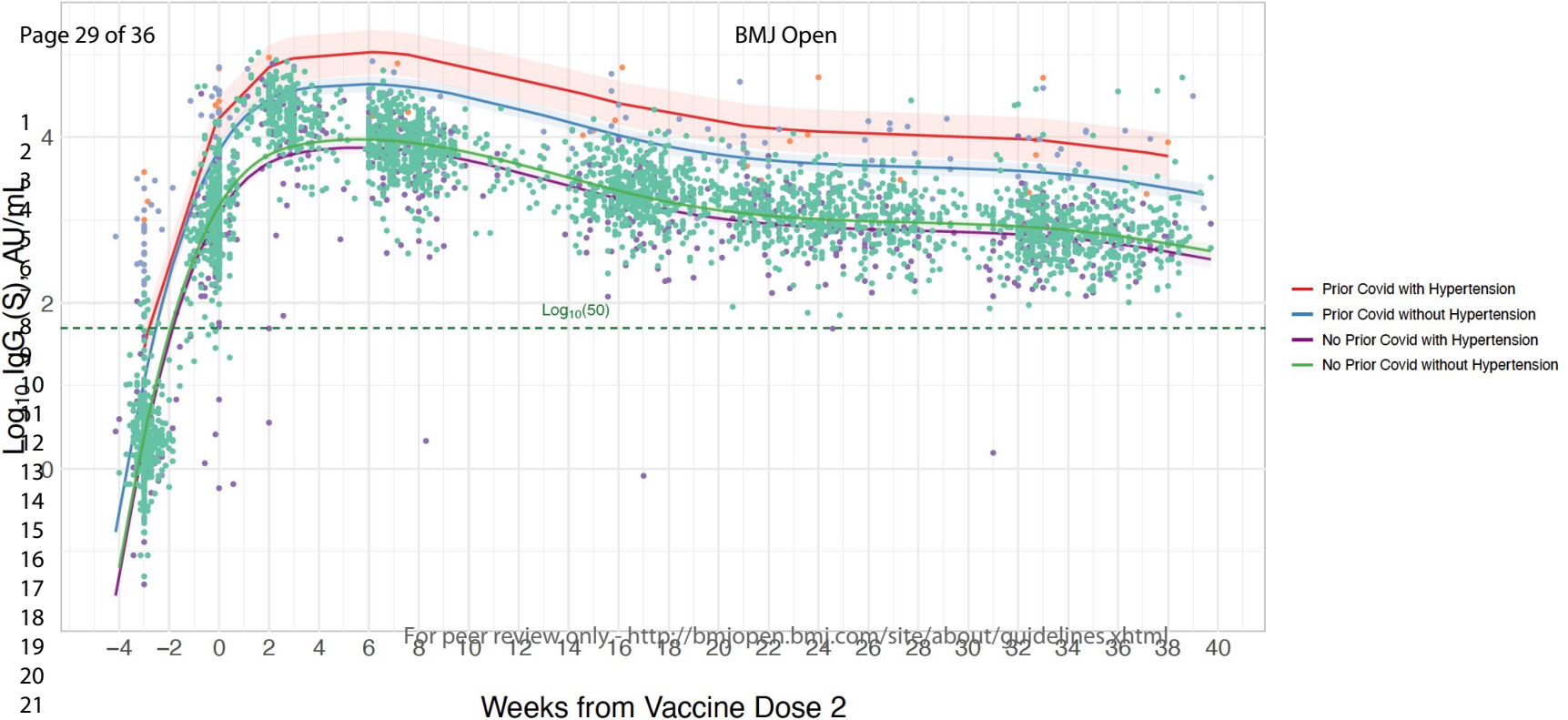
Figure 3. Longitudinal trajectory of IgG-S antibody levels following completed BNT162b2 vaccination by prior infection status and sex. Multivariable-adjusted longitudinal trajectories are shown for individuals with a history of prior COVID-19 infection for those without prior COVID-19 infection, including an interaction for sex. Longitudinal estimates with 95% confidence limits (shaded areas) are adjusted for age and hypertension.

Figure 4. Longitudinal trajectory of IgG-S antibody levels following completed BNT162b2 vaccination by prior infection and hypertension status. Multivariable-adjusted longitudinal trajectories are shown for individuals with a history of prior COVID-19 infection for those without prior COVID-19 infection, including an interaction for sex. Longitudinal estimates with 95% confidence limits (shaded areas) are adjusted for age and sex.









Determinants of the Longitudinal Antibody Response Following Initial mRNA Vaccination

Supplemental Material

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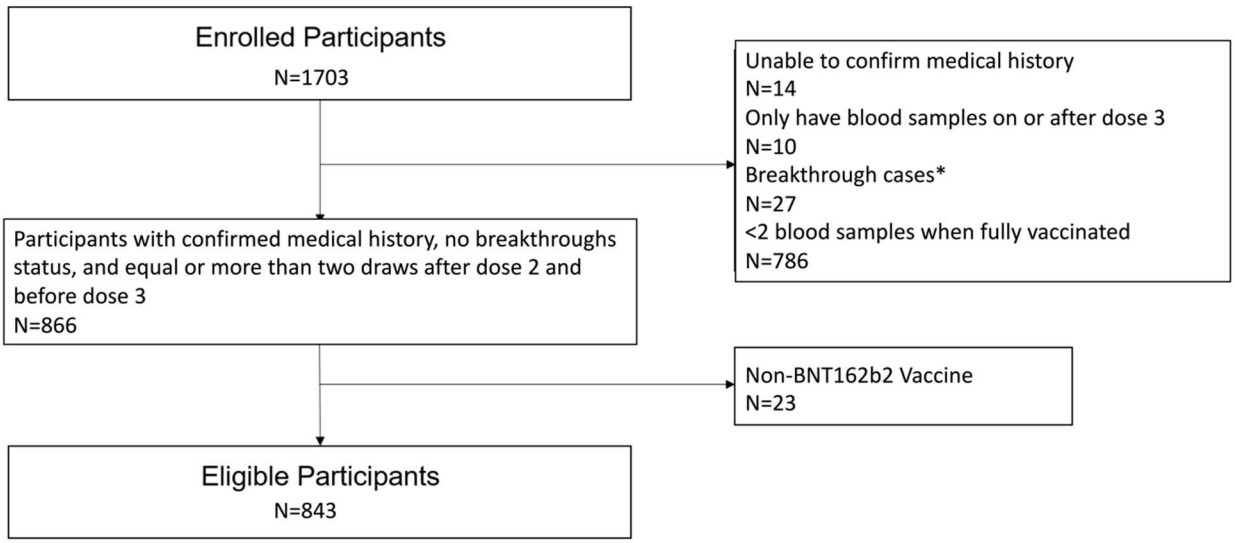
Supplemental Figure 1. Cohort Development Flow Diagram.



*Breakthrough cases had IgG N >=1.4 when measured fully vaccinated and before the third dose of vaccination, and the measurement of IgG N before is less than 0.4. We also excluded participant who only had one measurement (IgG N>=1.4 when fully vaccinated) and no prior covid-19 infection history.

Supplemental Table 1. Comparison of characteristics between the included and excluded study samples.

	Total Sample N=1689	Included N=843	Excluded N=846	P
Age in years, median [IQR]	39·9 [33·5, 51·1]	41·7 [35·2, 52·8]	38·0 [32·4, 49·5]	<0·001
Male sex, n (%)	537 (31·8)	256 (30·4)	281 (33·2)	0·229
Non-white race, n (%)	869 (51·5)	405 (48·0)	464 (54·8)	0·006
Hispanic ethnicity, n (%)	221 (13·1)	86 (10·2)	135 (16·0)	0·001
Obesity	250 (14·8)	103 (12·2)	147 (17·4)	0·004
Hypertension	241 (14·3)	128 (15·2)	113 (13·4)	0·315
Charlson comorbidity index	0·0 [0·0, 0·0]	0·0 [0·0, 1·0]	0·0 [0·0, 0·0]	0·006

Supplemental Table 2. Study sample characteristics.

	Total Sample	No Prior SARS-CoV-2 Infection	Prior SARS-CoV-2 Infection	P-Value*
N	843	784	59	
Age in years, median [IQR]	41·66 [35·19, 52·80]	41·89 [35·25, 53·00]	38·72 [34·93, 49·31]	0·169
Age in years, range	20·37-87·26	20·37-87·26	23·52-76·87	
Male sex, n (%)	256 (30·4)	239 (30·5)	17 (28·8)	0·903
Non-white race, n (%)	405 (48·0)	372 (47·4)	33 (55·9)	0·262
Hispanic ethnicity, n (%)	86 (10·2)	73 (9·3)	13 (22·0)	0·004
Obesity, n (%)	103 (12·2)	92 (11·7)	11 (18·6)	0·175
Hypertension, n (%)	128 (15·2)	122 (15·6)	6 (10·2)	0·355
Charlson comorbidity index, median [IQR]†	0·00 [0·00, 1·00]	0·00 [0·00, 1·00]	0·00 [0·00, 1·00]	0·572

*P-value comparing those with versus without prior SARS-CoV-2 infection.
†The Charlson comorbidity index weights the clinical conditions into a single score to predict 10-year survival: age, myocardial infarction, heart failure, peripheral vascular disease, stroke, dementia, chronic obstructive pulmonary disease, connective tissue disease, peptic ulcer disease, liver disease, diabetes mellitus, hemiplegia, chronic kidney disease, solid tumor, leukemia, lymphoma and AIDS.

Supplemental Table 3. Correlates of longitudinal anti-spike IgG antibody response following complete initial mRNA vaccination, stratified by prior SARS-CoV-2 infection status.

	No Prior SARS-CoV-2 Infection N=784			Prior SARS-CoV-2 Infection N=59		
	Beta*	SE	P	Beta*	SE	P
Age, year	-0·01	0·00	<0·001	-0·00	0·01	0·74
Male sex	-0·24	0·06	<0·001	-0·72	0·33	0·032
Hypertension	-0·23	0·08	0·005	0·96	0·50	0·06

*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable), in analyses adjusted for age, sex, and hypertension.

Supplemental Table 4. Association of prior SARS-CoV-2 infection with longitudinal anti-spike IgG antibody response following complete initial mRNA vaccination, stratified by age, sex, and hypertension status.

A.

	Age <42 years N=421			Age ≥42 years N=422		
	Beta*	SE	P	Beta*	SE	P
Prior SARS-CoV-2 infection	1·57	0·13	<0·001	1·93	0·19	<0·001

*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable), in analyses adjusted sex and hypertension.

B.

	Males N=256			Females N=587		
	Beta*	SE	P	Beta*	SE	P
Prior SARS-CoV-2 infection	1·35	0·20	<0·001	1·86	0·13	<0·001

*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable), in analyses adjusted for age and hypertension.

C.

	No Hypertension N=715			Hypertension N=128		
	Beta*	SE	P	Beta*	SE	P
Prior SARS-CoV-2 infection	1·61	0·11	<0·001	2·77	0·43	<0·001

*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable), in analyses adjusted for age and sex.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	8

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*9*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	13-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Demographic and clinical characteristics associated with variations in antibody response to BNT162b2 COVID-19 vaccination among healthcare workers at an academic medical center: a longitudinal cohort analysis

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Secondary Subject Heading:	Infectious diseases, Immunology (including allergy)

Keywords:	COVID-19, Hypertension < CARDIOLOGY, INFECTIOUS DISEASES

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Demographic and clinical characteristics associated with variations in antibody response to BNT162b2 COVID-19 vaccination among healthcare workers at an academic medical center: a longitudinal cohort analysis

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ABSTRACT

Objectives We sought to understand the demographic and clinical factors associated with variations in longitudinal antibody response following completion of 2-dose regiment of BNT162b2 vaccination.

Design This study is a 10-month longitudinal cohort study of healthcare workers and serially measured anti-spike protein IgG (IgG-S) antibody levels, using mixed linear models to examine their associations with participant characteristics.

Setting A large, multi-site academic medical center in Southern California, USA.

Participants A total of 843 healthcare workers met inclusion criteria including completion of an initial two-dose course of BNT162b2 vaccination, complete clinical history and at least 2 blood samples for analysis. Patients had an average age of 45±13 years, were 70% female, and 7% with prior SARS-CoV-2 infection.

Results Vaccine induced IgG-S levels remained in the positive range for 99·6% of individuals up to 10 months after initial 2-dose vaccination. Prior SARS-CoV-2 infection was the primary correlate of sustained higher post-vaccination IgG-S levels (partial-r2=0·133), with a 1·74±0·11 SD higher IgG-S response (P<0·001). Female sex (beta 0·27±0·06, P<0·001), younger age (0·01±0·00, P<0·001), and absence of hypertension (0·17±0·08P=0·003) were also associated with persistently higher IgG-S responses. Notably, prior SARS-CoV-2 infection augmented the associations of sex (-0·42 for male sex, P=0·08) and modified the associations of hypertension (1·17, P=0·001), such that infection-naïve individuals with hypertension had persistently lower IgG-S levels whereas prior-infected individuals with hypertension exhibited higher IgG-S levels that remained augmented over time.

Conclusions While the IgG-S antibody response remains in the positive range for up to 10 months following initial mRNA vaccination in most adults, determinants of sustained higher antibody levels include prior SARS-CoV-2 infection, female sex, younger age, and absence of hypertension. Certain determinants of the longitudinal antibody response appear significantly

modified by prior infection status. These findings offer insights regarding factors that may influence the 'hybrid' immunity conferred by natural infection combined with vaccination.

Keywords: SARS-CoV-2, longitudinal antibody response, sex differences, hypertension

Strengths and limitations of this study

- Evaluation of demographic and clinical characteristics associated with variable longitudinal antibody response following BNT162b2 vaccination.
- Among the longest follow up studies of COVID-19 vaccine associated humoral immune response
- Large, diverse study cohort.
- Prospective study design.
- Assessment of humoral, but not T-cell mediated antibody response.

INTRODUCTION

Exposure to SARS-CoV-2 or its subunits, via natural infection or vaccination, can elicit a humoral immune response that is measurable in the circulation and correlated with relative protection from future infections.[1-4] Recent studies have indicated that this quantifiable humoral response wanes over time – as soon as 3 to 6 months following either natural infection or initial administration of a SARS-CoV-2 vaccine.[5-7] While certain population subsets may experience more or less durable immunity from an initial natural or vaccine exposure, the demographic and clinical characteristics that may influence temporal variations in provoked humoral immune response currently remain unclear.[8]

Given lack of clarity regarding the factors that could promote accelerated versus delayed decline in acquired SARS-CoV-2 immunity, along with concern for immunocompromised persons at the highest risk for opportunistic infections, governments worldwide have made provisions to offer additional 'booster' vaccine doses.[9-11] Amidst rollout of the booster vaccinations, there remains equipoise regarding their appropriateness for individuals suspected of having more robust immunity following initial vaccination – including those recovered from prior SARS-CoV-2 infection and younger healthy persons. In fact, emerging data suggest that individuals who have been both fully vaccinated and previously infected with SARS-CoV-2 are likely to benefit from a 'hybrid immunity' that offers durable protection from infection in terms of both strength and longevity.[12-15]

To improve our understanding of the longitudinal immune response following initial SARS-CoV-2 vaccination – and the factors associated with variations in this response – we examined the demographic and clinical correlates of anti-spike IgG antibody (IgG-S) levels measured serially in a large cohort of fully vaccinated adults.

METHODS

Study sample

We conducted serial serological assays from a longitudinal cohort study of healthcare workers who received vaccination with Pfizer-BioNTech (BNT162b2) at our medical center in Southern California, with study design and sampling procedures detailed previously.[16] Briefly, participants completed surveys on medical history, exposures, and symptoms at baseline and at serial timepoints over the course of the study. All healthcare workers, including those recovered from prior COVID-19 infection, were advised to receive a full vaccination course including 2 doses of mRNA vaccine according to local department of health and institutional policies. History of SARS-CoV-2 infection prior to vaccination was determined based on self-report along with adjudication of medical records or confirmed presence of antibodies targeting the viral nucleocapsid protein [IgG(N)]; given that the nucleocapsid protein is not produced by mRNA vaccination, elevated IgG(N) antibodies are considered indicative of prior infection. Participants were excluded if they received a vaccine other than BNT162b2, their SARS-CoV-2 infection status could not be confirmed, they developed a breakthrough infection any time after 14 days following second dose, or they did not provide at least 2 blood samples for serology following completion of their second vaccine dose. All participants provided written informed consent for all protocols, which were reviewed and approved by the Cedars-Sinai institutional review board.

Serology

Serological assays for antibodies to the receptor binding domain (RBD) of the S1 subunit of the viral spike protein [IgG (S-RBD)], and IgG(N) were performed using the Abbott SARS-CoV-2 IgG II assay and SARS-CoV-2 IgG assay, respectively (Abbott Labs, Abbott Park, IL). Antibody levels were measured from plasma samples collected at the following time points: before or up to 3 days after dose 1; within 7 to 21 days after dose 1; within 7 to 21 after dose 2; and then at 8, 16, 24, 32, and 40 weeks after dose 2. We considered an IgG(N) signal to cutoff (S/C) index

of ≥1.4 as denoting definitive seropositive status due to prior SARS-CoV-2 exposure, based on a previously established thresholds.[17]

Statistical analyses

For descriptive statistics, we used analysis of variance to test for differences between continuous normally distributed variables, Kruskal-Wallis rank sum tests for non-normal continuous variables, and chi squared test for categorical variables. We used mixed-effect linear modeling to estimate the mean and 95% confidence interval of log(10)IgG-S levels in relation to time since the date of complete vaccination (i.e. dose 2), with time expressed using natural cubic splines. For longitudinal modeling, we used the Akaike Information Criterion (AIC) as a measure of best fit to select the optimal number of knots, which was optimized when using 4 knots placed at the 5th, 35th, 65th, and 95th percentiles. We treated repeated measures for each participant as random effect and additionally adjusted for age, sex, race, ethnicity, obesity, hypertension, and the Charlson comorbidity index[18] calculated based on the combination of information collected from medical history surveys and the electronic health record.[16 19] In secondary analyses, we repeated multivariable-adjusted mixed-effect regression analyses including multiplicative interaction terms for any significantly associated demographic or clinical variables, to assess for potential effect modification of the anticipated relation between prior SARS-CoV-2 infection on longitudinal log(10)IgG-S trajectory. We conducted all statistical analyses using R (v4.1.1) and considered statistical significance as a two-tailed P value less than 0·05.

Patient and public involvement

Patients and the public were not involved in the development of this study.

RESULTS

A total of 1,703 healthcare workers were enrolled in the source cohort between November 30, 2020 and November 11, 2021. From the source cohort, we excluded from the present analysis a total of n=860 individuals based on the following criteria: SARS-CoV-2 infection status could not be confirmed (n=14), developed a breakthrough infection (n=27), did not provide at least 2 blood samples for serology following completion of their second vaccine dose and prior to a 3rd vaccine dose (n=796), or did not receive the BNT162b2 vaccine (n=23). After exclusions, the final cohort for the present analysis included N=843 individuals (**Figure 1**). Of these, n=59 (7.0%) had a history of SARS-CoV-2 infection all of whom survived index infection (with only 5% requiring hospitalization) and were considered to have recovered successfully (without persistent or recurrent symptoms). Among participants for whom the date of first positive SARS-CoV-2 PCR was available (n=28), the average time from prior infection to first vaccine dose was 139 days (range 14-292 days). The demographic and clinical characteristics of our study sample (**Table 1**) revealed no clinically important differences in age, sex, or comorbidities between individuals with and without prior infection. Slightly more individuals with compared to without a history of COVID-19 reported working on a hospital ward where COVID-19 patients were cared for (32.2% vs 18.1%, P=0.013). Differences between included and excluded, as well as between older and younger participants are displayed in **Supplemental Tables 1** and **2**.

In spline analyses of the longitudinal trajectory of response in log(10)IgG-S levels following vaccination, we observed that 99·6% of all healthcare worker participants had repeated values that remained within the positive reference range of ≥ log(10)50 AU/mL over the entire follow-up period of up to 40 weeks (**Figure 2**). The number of blood samples available each week, stratified by prior COVID-19 status is presented in **Supplemental Figure 1**. In multivariable-adjusted models examining demographic and clinical correlates of longitudinal IgG-S levels, we found that prior SARS-CoV-2 infection was associated with substantially higher

antibody levels with prior infected individuals exhibiting an almost 1·7-fold higher standard deviation in log(10)IgG-S levels compared to never infected individuals (**Table 2**). Whereas younger age and female sex were also significantly associated with higher IgG-S levels over the duration of the study period, prior SARS-CoV-2 infection was the predominant determinant with the largest model partial r2 value of 0·134. These results indicate that 13·4% of the observed variation in longitudinal IgG-S levels was attributable to prior infection status even after accounting for other covariates in the model that include age, sex, race, ethnicity, hypertension, obesity, and the Charlson comorbidity burden index.

In secondary analyses, we found that the interaction between age and prior infection status on longitudinal IgG-S levels was non-significant (beta 0.37, P=0·10, **Figure 3, Supplemental Table 3**) although, in exploratory analyses stratified by prior infection status, older age was significantly associated with lower IgG-S response among infection-naïve individuals whereas no significant age-based association was seen in prior-infected individuals (**Supplemental Table 4**). This is similar to the interaction of male sex with prior infection (beta -0.42, P=0·08, **Figure 4, Supplemental Table 3**), with stratified analysis demonstrating that male compared to female sex was associated with greater magnitude of difference in IgG-S level in prior infected (beta -0·72 [se 0·33], P=0·032) compared to never infected individuals (beta -0·24 [se 0·06], P<0·001) (**Supplemental Table 4)**. Notably, we observed a significant interaction between hypertension and prior infection (beta 1.17, P=0·001, **Figure 5, Supplemental Table 3**), with hypertension significantly associated with lower IgG-S levels in never infected persons (beta -0·23 [se 0·08], P=0·005) while concurrently related to higher IgG-S levels in prior infected individuals (beta 0·96 [se 0·50], P=0·06) in stratified analysis (**Supplemental Table 4**). Similarly, age and sex demonstrated a significant interaction, such that older age (above the median cohort age of 42 years) was associated with lower antibody levels among males compared to females (**Supplemental Table 3**). Analyses stratified by age,

sex, and prior infection status demonstrated concordant results (**Supplemental Table 5**). The number of blood samples available each week, stratified by age, sex and hypertensive status are presented in **Supplemental Figure 1**.

DISCUSSION

From our study of repeated serological measures performed in a large cohort with two-dose initial BNT162b2 vaccination, there were several key findings. First, we found that the vast majority of adults in our cohort maintained appropriate elevations of IgG-S antibody levels within the positive reference range up to 10 months following initial complete vaccination. Second, the primary differentiator of antibody response trajectory was prior SARS-CoV-2 infection, with a relatively fixed magnitude of variance that lasted throughout the follow up period. Finally, correlates of persistently higher longitudinal antibody response level included female sex, younger age, and absence of hypertension in analyses adjusting for race, ethnicity, and comorbidities. Intriguingly, the longitudinal effect of prior infection status was differentially modified by these associations – particularly hypertension status.

Extending from prior studies,[5 6] we repeated serological measures up to 10 months following initial SARS-CoV-2 vaccination in a large cohort of adults who receive their BNT162b2 vaccinations according to the standardized 2-dose schedule. While observing an initial peak and then steady decline in the absolute levels of IgG-S antibody response, as seen in other studies, we also found a relatively consistent pattern of longitudinal response that almost invariably involved levels remaining in the positive range during the follow-up period. Specifically, we found that the average trajectory of response in IgG-S antibody levels peaks within the first 2 to 8 weeks after the second vaccine dose and then declines towards a relative plateau – seen on the log10 scale – that lasts up to 40 weeks. Notwithstanding continued reductions in the absolute IgG-S antibody levels, the relative plateau on the log scale signals an attenuation in the rate of decline and is consistent with the longitudinal patterns of post-vaccination antibody titer response that has been reported for other viruses (e.g. influenza) and predicted for SARS-CoV-2.[20-22] Although the threshold of 50 AU/mL for absolute IgG-S antibody levels is validated with 99·5% specificity for detecting antibodies specific to the SARS-CoV-2 spike protein, and

the exact quantitative thresholds that may correspond to effective immunity remains unclear, a relative plateau in the log10 scale presence of IgG-S offers some assurance of continued memory B cell activation potentially indicative of an even broader immunological reserve.

In addition to the overall trajectory common to most participants, we found that the primary and persistent differentiator of antibody response trajectory was prior SARS-CoV-2 infection. Extending from prior studies that examined serological responses up to 6 months after SARS-CoV-2 vaccination,[5] we observed a relatively fixed magnitude of difference in provoked IgG-S levels – consistently higher in prior infected compared to never infected individuals – persisting beyond 10 months. The absence of any indication that this difference is narrowing suggests that the 'hybrid' immunity obtained from the combination of natural infection and vaccination is likely to endure over time – a phenomenon consistent with recent findings of dynamic memory B cell activation and clonal turnover in individuals exposed to both natural infection and vaccine.[12] Furthermore, and intriguingly, prior infected individuals had persistently elevated post-vaccine antibody levels that did not differ by age – indicating minimal influence of age-related humoral deficiency on the 'hybrid' or dose-boosted effect.[23 24] We recommend that the age-based results of our analyses be interpreted with caution, given the relatively younger overall age range of our cohort. Additional studies in cohorts with older age ranges are needed to assess the generalizability of our findings. By contrast, the female advantage in antibody response to SARS-CoV-2 vaccination has been previously reported[6 25] and in our cohort appeared accentuated by prior infection such that previously infected females tended to exhibit the most pronounced as well as persistently elevated antibody response. Females are known to generate antibody responses to a variety of viral vaccines that are almost twice as high as the responses seen in males.[26] Augmentation and persistence of this sex difference in the setting of 'hybrid' SARS-CoV-2 exposure points to a female advantage in at least humoral immunity that

could represent a mechanistic contributor to the female advantage seen in COVID-19 related outcomes.

Our results regarding the associations of hypertension with longitudinal antibody response are especially notable. Extending from prior studies focused on initial post-vaccine effects,[27 28] we found that presence of hypertension was associated with an overall lower level antibody response that was consistent over time and persisted for up to 10 months. Intriguingly, we also found that among persons with prior SARS-CoV-2 infection, the association of hypertension status on longitudinal IgG-S antibody response was reversed. In effect, longitudinal antibody levels are profoundly increased among hypertensive participants with prior COVID-19 compared to without prior COVID-19. Previous studies have demonstrated a more robust antibody response following native infection among hypertensive individuals – attributed to a combination of increased sympathetic drive and an underlying inflammatory state serving to enhance immune activation.[29 30] These same factors have been hypothesized as contributors to the greater mortality risk seen among hypertensive COVID-19 patients. In light of the lower antibody response to vaccination seen in hypertensives overall, the paradoxically higher response seen in hypertensives with prior COVID-19 is similar to the trend seen for older-aged individuals with prior infection. In both situations, a pre-existing relative deficiency in immune reserve is superseded by the effects of having been directly exposed to and then recovered from COVID-19. Importantly, these effects appear to persist in the population over time.

Several limitations of this study merit consideration. First, all participants received the Pfizer-BioNTech (BNT162b2) vaccine, limiting generalizability to other vaccines, although variable waning of antibody levels following other SARS-CoV-2 vaccines has been described.[8] All participants were also healthcare workers with the greater risk for repeated SARS-CoV-2 exposure via the work environment, which may or may not have influenced their long-term antibody response. There also exists potential bias in the study population, as not all

participants provided longitudinal serology data, although there were negligible clinically meaningful differences between those with and without adequate serology data for inclusion. Importantly, all prior infected individuals in our study were not only survivors of COVID-19 but were predominantly less severely affected with only 5% requiring hospitalizations, all of which lasted less than 5 days, and none reporting continued or recurrent symptoms following recovery from the index infection. This issue is particularly important to consider when interpreting interaction analyses, as a provoked humoral immune response that is augmented to a level that is sufficient for countering infection is likely different from an exaggeration in response that may contribute to end-organ dysfunction or continued symptoms. Additionally, the majority of prior infected individuals had pre-vaccination antibody levels measured within a similar range to infection naïve individuals, likely a result of the antibody decay that has been observed in prior studies of longitudinal antibody response following natural infection.[31] Further studies are needed to assess longitudinal antibody response to vaccination administered within shorter-time frames following prior infection. To accommodate healthcare worker availability for participation, plasma samples were collected within a 7-21 day period after each vaccine dose and the differences in timing within these sampling windows may have contributed to some variation in results. Because viral variant testing was not routinely conducted for participant samples, data on which variants contributed to confirmed infections were not available for analyses. We also do not address non-humoral related immune protection, which may protect or predispose to future infections.

In summary, our findings indicate that completion of a two-dose mRNA vaccine regimen provokes an IgG-S antibody response that is not only enhanced but also persistent among individuals with prior SARS-CoV-2 infection when compared to those without prior infection. Further, our results demonstrate potential sex and hypertension specific variations in the longitudinal response to single vs dual antigenic exposure that may guide more tailored

assessments of individual-level risks for future infection. In particular, the role of hypertension as a potential potent modifier of antibody response, with divergent post-vaccination effects between those with and without prior infection, may reflect key differences in physiologically mediated immune response among those with and without high blood pressure. These findings may allow for allocation of still limited vaccine resources by targeting individuals most likely to benefit from additional vaccine doses.

Contributors

JEE contributed via Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, and Project Administration. SJ contributed via Conceptualization, Resources, Data Curation, and Writing - Review & Editing. YL and MW contributed via Methodology, Formal Analysis, Writing - Original Draft, Writing - Review & Editing, and Visualization. BW, YHK, BK, TW, TTN, and MA contributed via Resources and Data Curation. BC contributed via Formal Analysis, Writing - Review & Editing, and Supervision. PGB, NS, and MD contributed via Validation, Investigation, and Writing - Review & Editing. JCP contributed via Methodology, Investigation, and Writing - Review & Editing. ECF contributed via Investigation and Resources. JLS, HSG, PC, and SCJ, contributed via Investigation, Resources, and Writing - Review & Editing. MJ contributed via Investigation, Resources, Writing - Review & Editing, and Funding Acquisition. SS, JFB, JEVE, MBM, MA, and GYM contributed via Investigation and Writing - Review & Editing. JGB contributed via Investigation, Writing - Review & Editing, Project Administration, and Funding Acquisition. DPBM contributed via Investigation, Writing - Review & Editing, and Project Administration. SC and KS Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project Administration, and Funding Acquisition. The corresponding authors attest that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. The manuscript's guarantors (JEE, SC, KS) affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Competing interests

JCP, ECF, and JLS work for Abbott Diagnostics, a company that performed the serological assays on the biospecimens that were collected for this study. The remaining authors have no disclosures to report.

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Ethics approval

This study was approved by the Cedars-Sinai Institutional Review Board (IRB) (CORALE Study00000621). All participants provided written informed consent for all protocols.

Data availability statement

Due to the sensitive nature of the data collected for this study, requests to access the dataset from qualified researchers trained in protocols on the protection of human subjects may be sent to Cedars-Sinai Medical Center at biodatacore@cshs.org.

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Table 1. Study sample characteristics.

	Total Sample	No Prior SARS-CoV-2 Infection	Prior SARS-CoV-2 Infection	P-Value*
N	843	784	59	
Age in years, median [IQR]	41·66 [35·19, 52·80]	41·89 [35·25, 53·00]	38·72 [34·93, 49·31]	0·169
Age in years, range	20·37-87·26	20·37-87·26	23·52-76·87	
Male sex, n (%)	256 (30·4)	239 (30·5)	17 (28·8)	0·903
Non-white race, n (%)	405 (48·0)	372 (47·4)	33 (55·9)	0·262
Hispanic ethnicity, n (%)	86 (10·2)	73 (9·3)	13 (22·0)	0·004
Obesity, n (%)	103 (12·2)	92 (11·7)	11 (18·6)	0·175
Hypertension, n (%)	128 (15·2)	122 (15·6)	6 (10·2)	0·355
Charlson comorbidity index, median [IQR]†	0·00 [0·00, 1·00]	0·00 [0·00, 1·00]	0·00 [0·00, 1·00]	0·572
Work Environment‡				
ICU, COVID-19 unit	135 (16·1)	126 (16·2)	9 (15·3)	1·00
ICU, non-COVID-19 unit	133 (15·9)	129 (16·5)	4 (6·8)	0·073
Ward, COVID-19 unit	160 (19·1)	141 (18·1)	19 (32·2)	0·013
Ward, non-COVID-19 unit	204 (24·3)	193 (24·7)	11 (18·6)	0·37
Emergency Department / Urgent care	98 (11·7)	94 (12·1)	4 (6·8)	0·315
Outpatient clinic	215 (25·6)	206 (26·4)	9 (15·3)	0·082
Office	129 (15·4)	119 (15·3)	10 (16·9)	0·873
Work from home	61 (7·3)	57 (7·3)	4 (6·8)	1·00
Other	185 (22·1)	177 (22·7)	8 (13·6)	0·142
Unknown	74 (8·8)	71 (9·1)	3 (5·1)	0·423

*P-value comparing those with versus without prior SARS-CoV-2 infection.
†The Charlson comorbidity index weights the clinical conditions into a single score to predict 10-year survival: age, myocardial infarction, heart failure, peripheral vascular disease, stroke, dementia, chronic obstructive pulmonary disease, connective tissue disease, peptic ulcer disease, liver disease, diabetes mellitus, hemiplegia, chronic kidney disease, solid tumor, leukemia, lymphoma and AIDS.
‡Participant provided work environment. Participants could select multiple environments if they worked in more than one location.

Table 2. Clinical and demographic correlates of longitudinal anti-spike IgG antibody response following complete initial mRNA vaccination.

	Beta*	SE	P	Partial r2
Prior SARS-CoV-2 infection	1·74	0·11	<0·001	0·134
Age, year	-0·01	0·00	<0·001	0·016
Male sex	-0·27	0·06	<0·001	0·013
Non-white race	-0·00	0·06	0·99	0·000
Hispanic ethnicity	0·02	0·10	0·80	0·000
Obesity	0·03	0·09	0·77	0·000
Hypertension	-0·17	0·08	0·041	0·003
Charlson comorbidity index	-0·02	0·03	0·56	0·000

*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable).

Figure 1. Cohort development flow diagram

Figure 2. Longitudinal trajectory of IgG-S antibody levels following completed BNT162b2 vaccination

Multivariable-adjusted longitudinal trajectories are shown for individuals with a history of prior COVID-19 infection (orange line) for those without prior COVID-19 infection (green line). Longitudinal estimates with 95% confidence limits (shaded areas) are adjusted for age, sex, and hypertension.

Figure 3. Longitudinal trajectory of IgG-S antibody levels following completed BNT162b2 vaccination by prior infection status and age

Multivariable-adjusted longitudinal trajectories are shown for individuals with a history of prior COVID-19 infection for those without prior COVID-19 infection, including an interaction for age (above vs below median cohort age). Longitudinal estimates with 95% confidence limits (shaded areas) are adjusted for sex and hypertension.

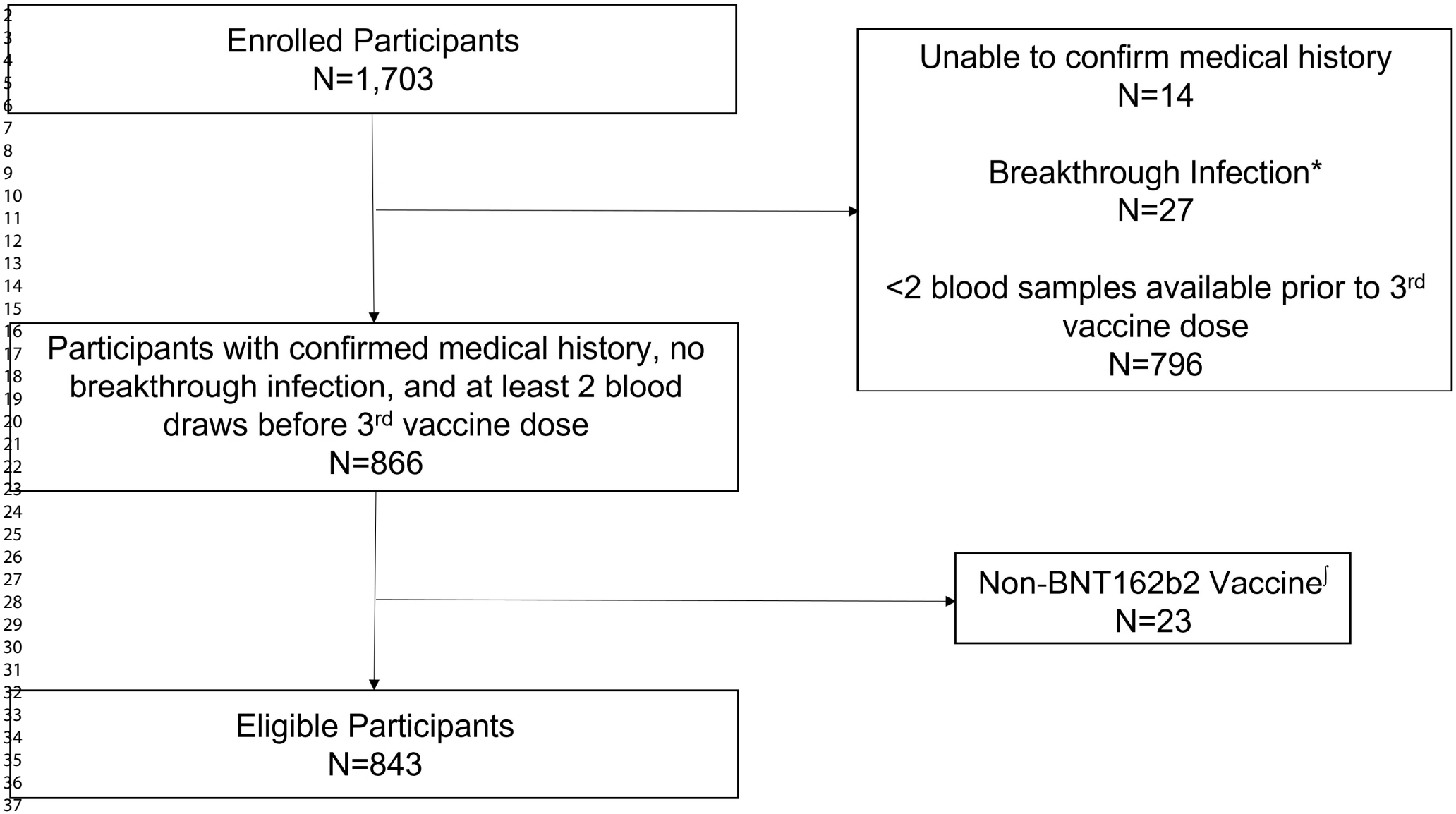
Figure 4. Longitudinal trajectory of IgG-S antibody levels following completed BNT162b2 vaccination by prior infection status and sex

Multivariable-adjusted longitudinal trajectories are shown for individuals with a history of prior COVID-19 infection for those without prior COVID-19 infection, including an interaction for sex. Longitudinal estimates with 95% confidence limits (shaded areas) are adjusted for age and hypertension.

Figure 5. Longitudinal trajectory of IgG-S antibody levels following completed BNT162b2 vaccination by prior infection and hypertension status

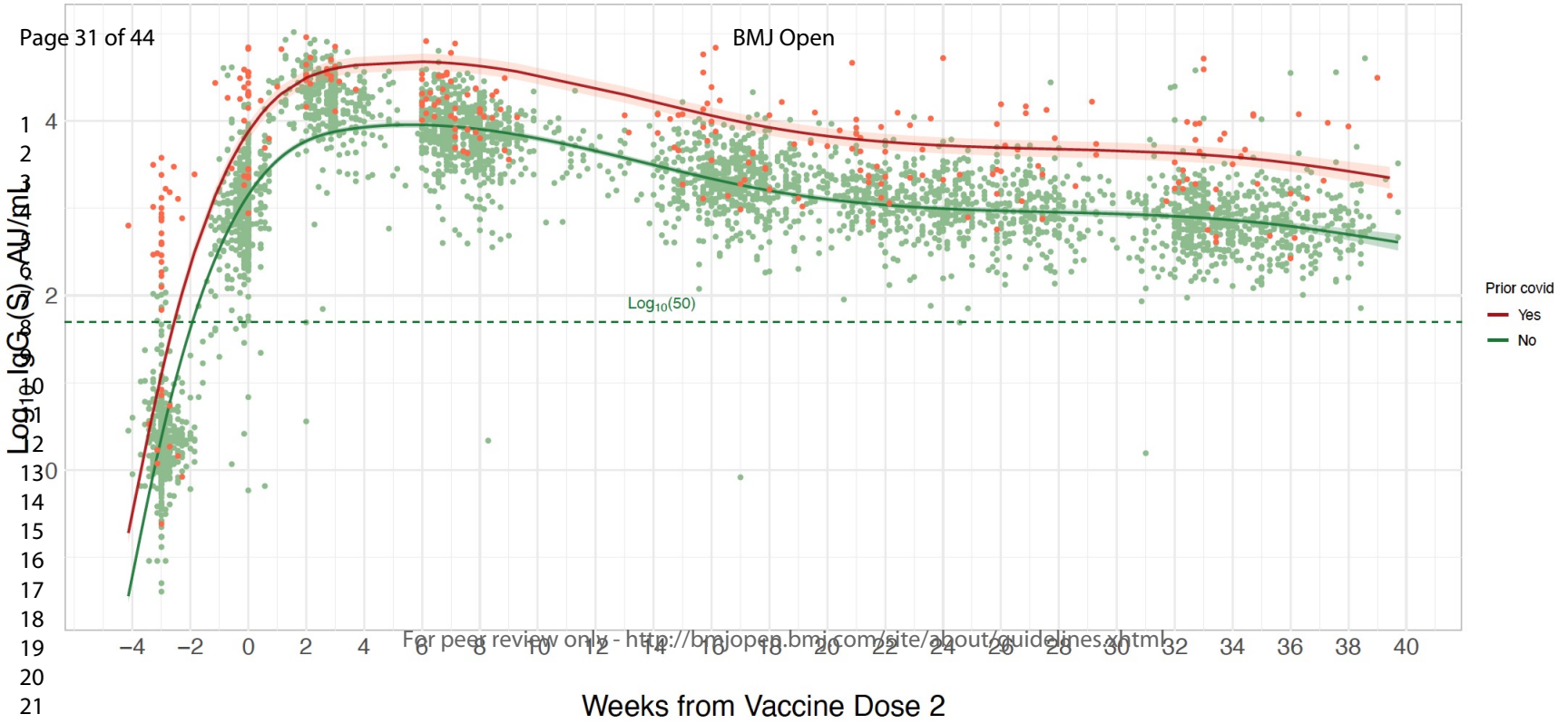
Multivariable-adjusted longitudinal trajectories are shown for individuals with a history of prior

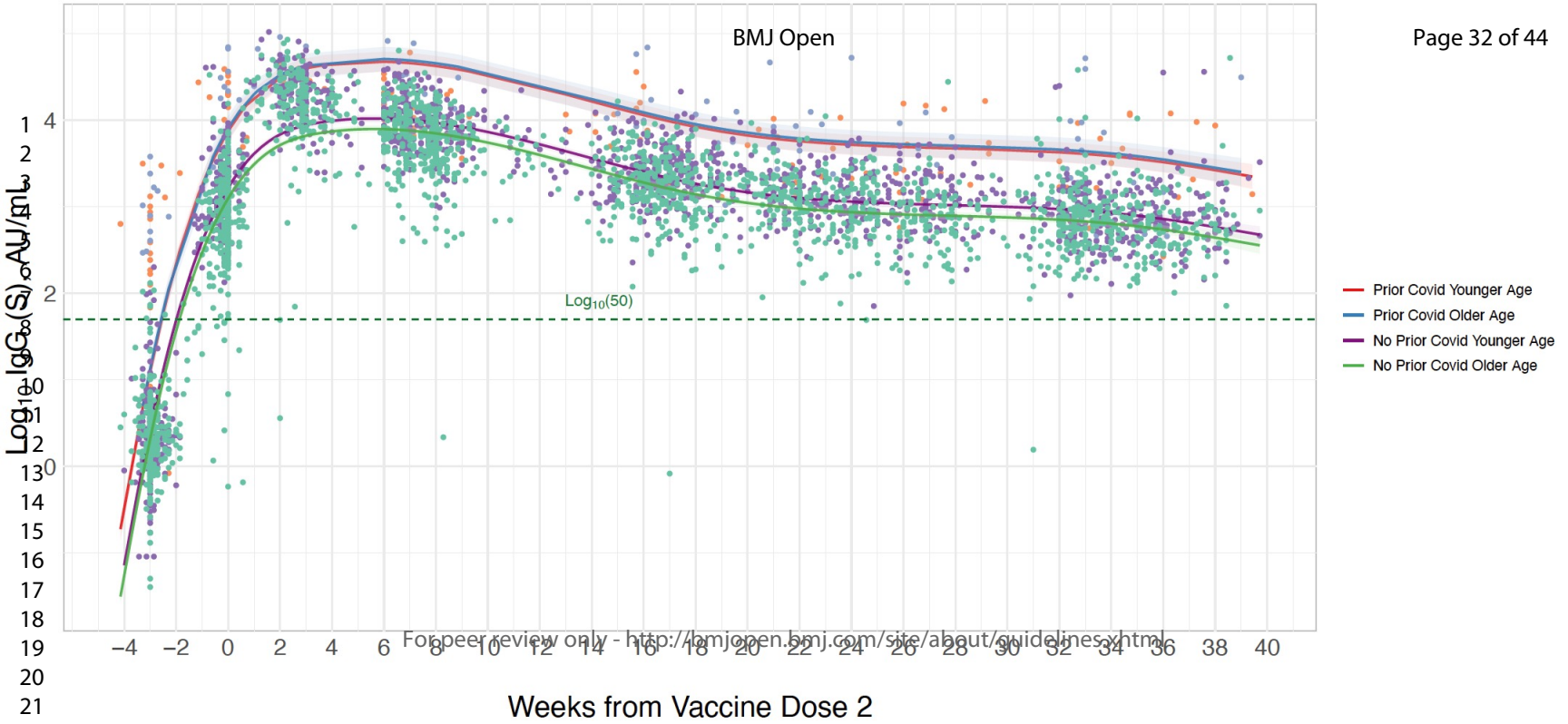
COVID-19 infection for those without prior COVID-19 infection, including an interaction for sex. Longitudinal estimates with 95% confidence limits (shaded areas) are adjusted for age and sex.

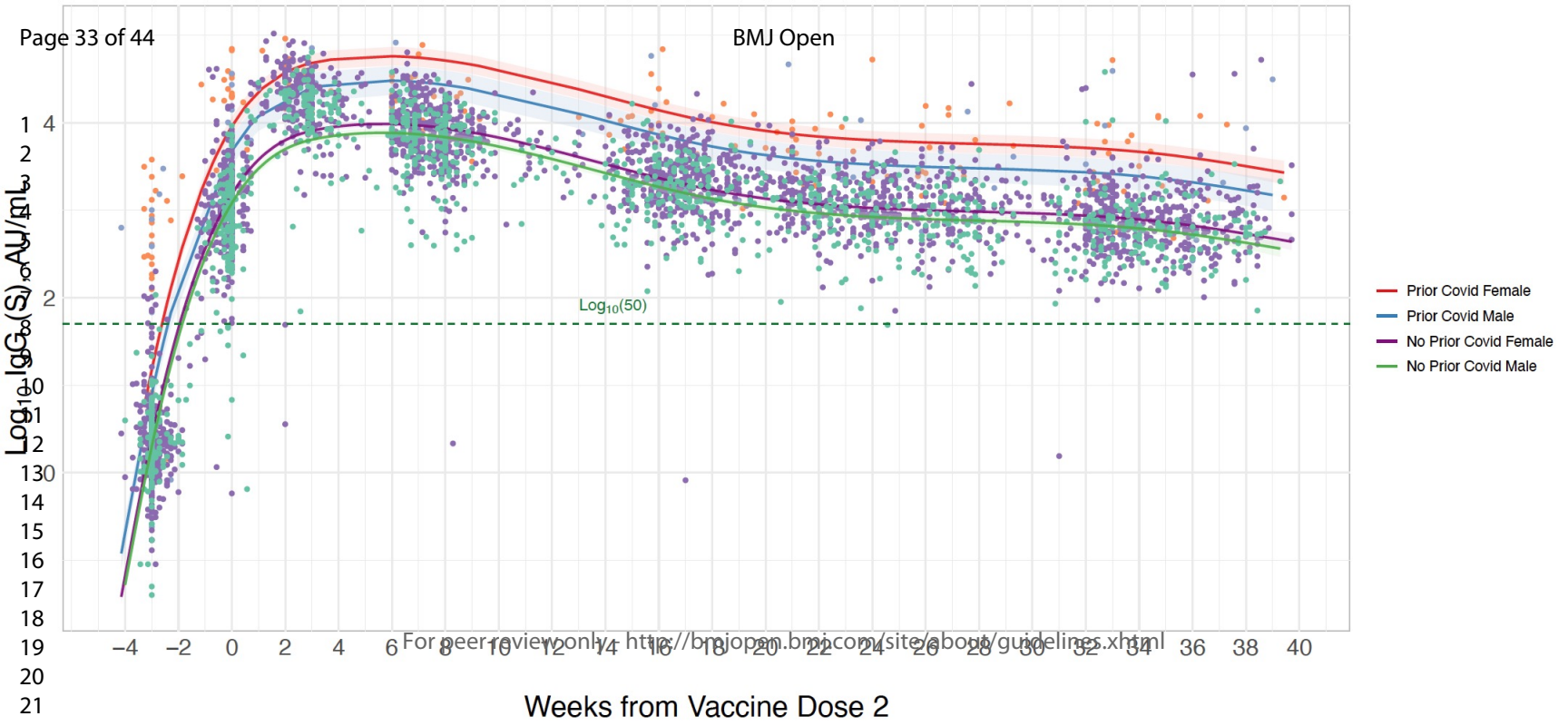


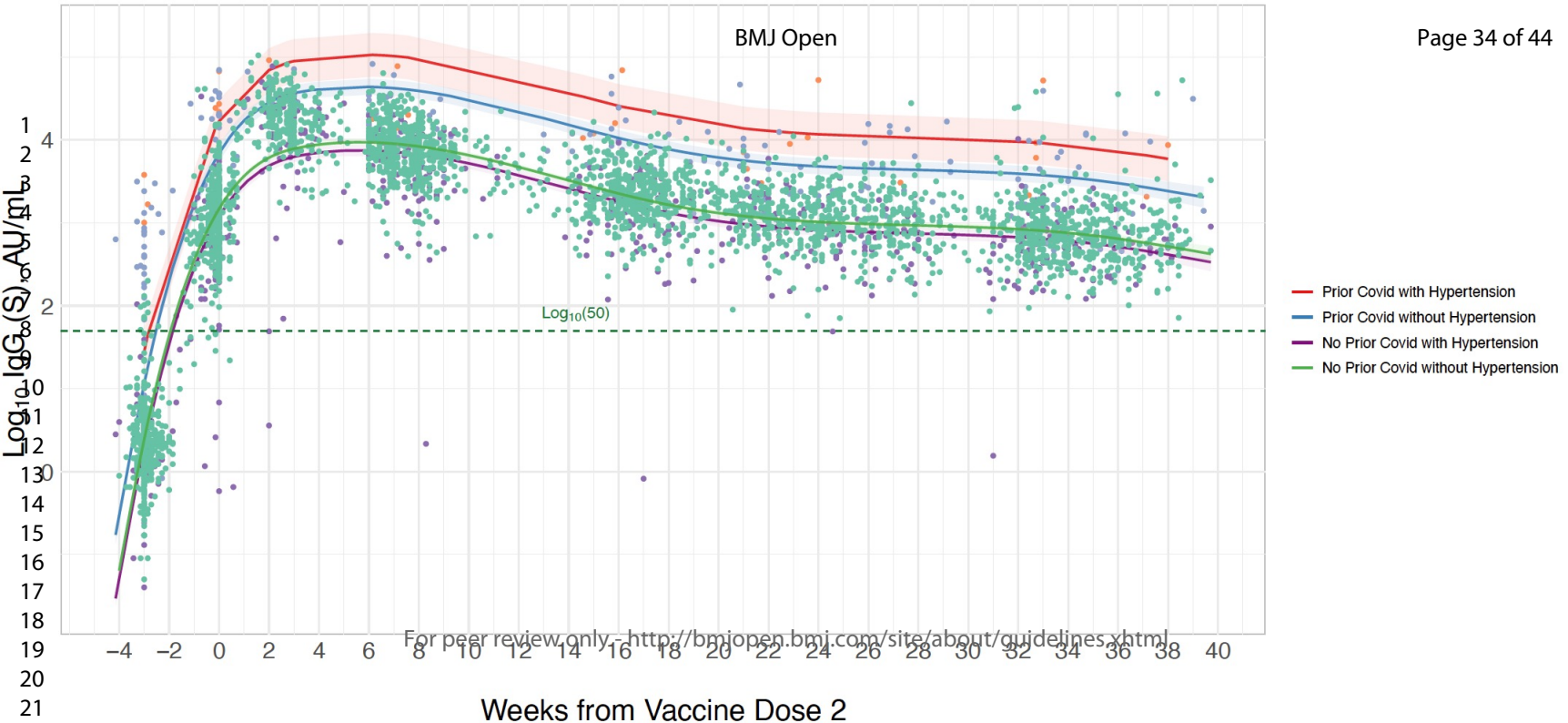
*Breakthrough cases defined as IgG(N) ≥1.4 when measured after receiving 2 mRNA vaccine doses and prior to a 3rd dose, with prior IgG(N) <0.4 or no history of prior COVID-19 infection.

∫ Participants who received any vaccine other than BNT162b2.









Longitudinal Cohort Analysis of Demographic and Clinical Characteristics Associated with Variations in Antibody Response to BNT162b2 Vaccination Among Healthcare Workers at an Academic Medical Center

Supplemental Material

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Supplemental Table 1: Comparison of characteristics between the included and excluded study samples.

	Total Sample N=1703	Included N=843	Excluded N=860	P
Age in years, median [IQR]	39.90 [33.59, 51.06]	41.7 [35.2, 52.8]	38.01 [32.41, 49.51]	<0.001
Male sex, n (%)	539 (31.7)	256 (30.4)	283 (32.9)	0.283
Non-white race, n (%)	879 (51.6)	405 (48.0)	474 (55.1)	0.004
Hispanic ethnicity, n (%)	224 (13.2)	86 (10.2)	138 (16.0)	<0.001
Obesity	252 (14.8)	103 (12.2)	149 (17.3)*	0.004
Hypertension	243 (14.3)	128 (15.2)	115 (13.4)*	0.318
Charlson comorbidity index†	0.00 [0.00, 0.00]	0.0 [0.0, 1.0]	0.00 [0.00, 0.00]*	0.009

*The data shown are for the 846 excluded participants who had medical history data available for ascertaining these clinical characteristics (i.e. obesity, hypertension, and Charlson comorbidity index).

†The Charlson comorbidity index weights the clinical conditions into a single score to predict 10-year survival: age, myocardial infarction, heart failure, peripheral vascular disease, stroke, dementia, chronic obstructive pulmonary disease, connective tissue disease, peptic ulcer disease, liver disease, diabetes mellitus, hemiplegia, chronic kidney disease, solid tumor, leukemia, lymphoma and AIDS.

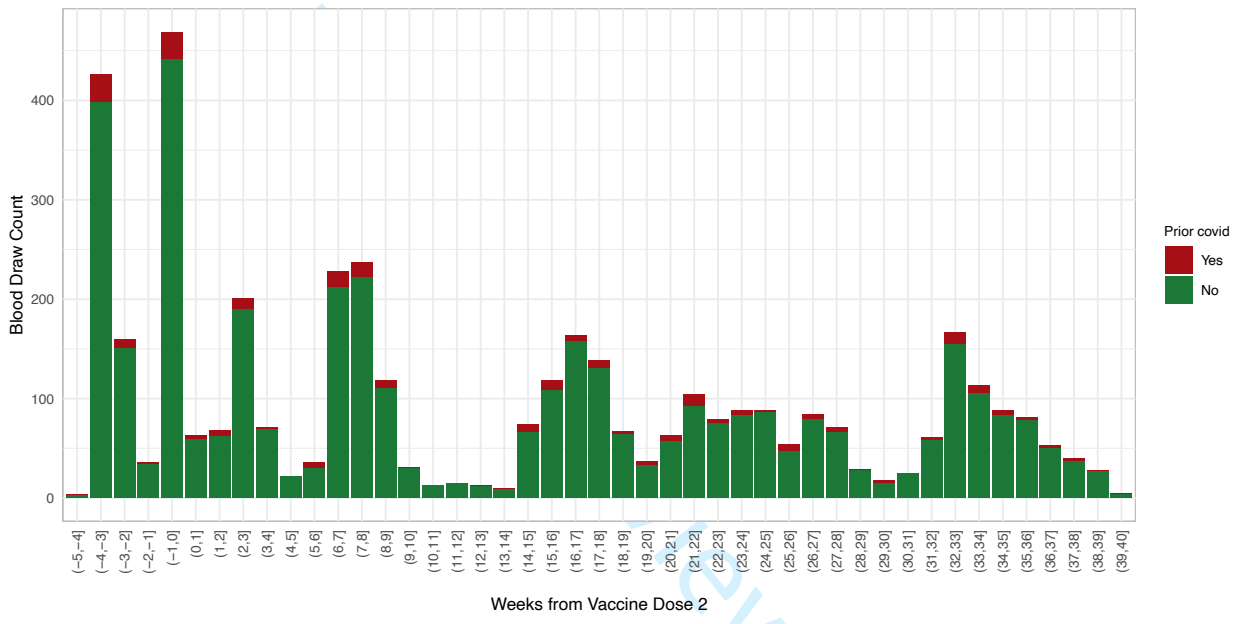
Supplemental Table 2. Comparison of characteristics between the older and younger study participants.

	Total Sample N=843	Younger Age* N=421	Older Age* N=422	P
Age in years, median [IQR]	41.66 [35.19, 52.80]	35.19 [31.55, 38.02]	52.80 [46.66, 62.25]	<0.001
Male sex, n (%)	256 (30.4)	105 (24.9)	151 (35.8)	0.001
Non-white race, n (%)	405 (48.0)	224 (53.2)	181 (42.9)	0.003
Hispanic ethnicity, n (%)	86 (10.2)	59 (14.0)	27 (6.4)	<0.001
Obesity	103 (12.2)	43 (10.2)	60 (14.2)	0.095
Hypertension	128 (15.2)	21 (5.0)	107 (25.4)	<0.001
Charlson comorbidity index	0.00 [0.00, 1.00]	0.00 [0.00, 0.00]	0.00 [0.00, 1.00]	<0.001

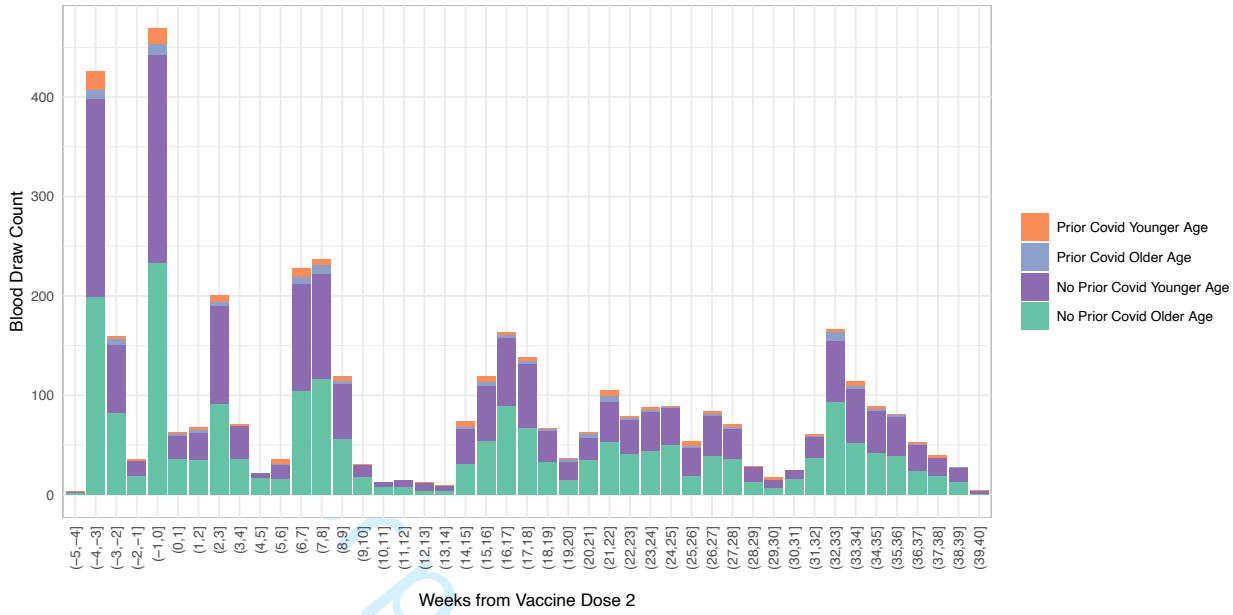
*Age definition based on if participant was younger or older in age than the median cohort age of 41.7 years.

Supplemental Figure 1. Number of available blood samples at each time point, stratified by prior COVID-19 status A) alone, or in combination with B) age, C) sex, and D) hypertension.

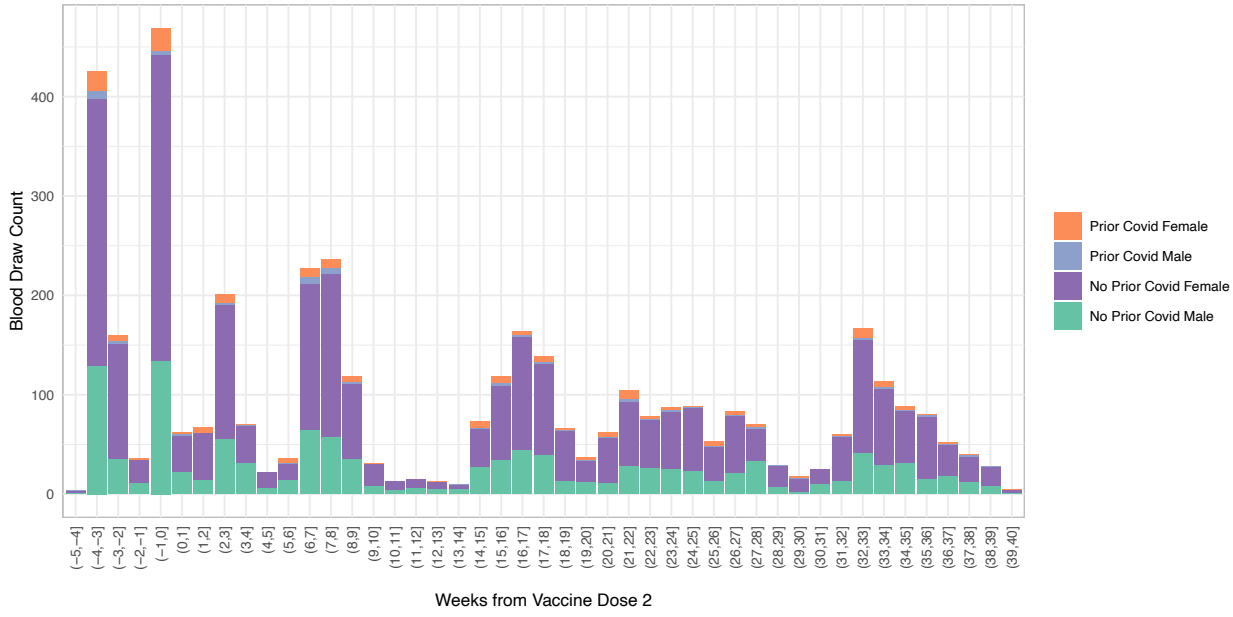
A.



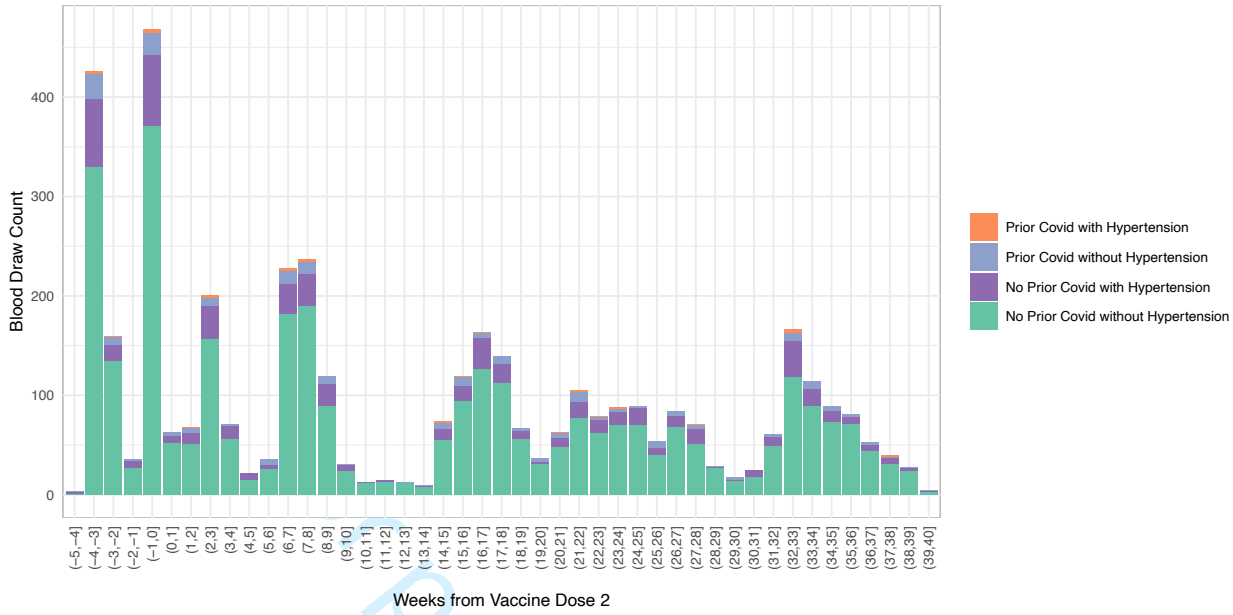
B.



C.



D.



Supplemental Table 3. Clinical and demographic correlates of longitudinal anti-spike IgG antibody response following complete initial mRNA vaccination, including interaction terms for A) age and prior SARS-CoV-2 infection, B) sex and prior SARS-CoV-2 infection, C) hypertension and prior SARS-CoV-2 infection, and D) age and sex.

A.

	Beta*	SE	P	Partial r2
Prior SARS-CoV-2 infection	1.58	0.14	<0·001	0.069
Older age†	-0.29	0.06	<0·001	0·016
Prior SARS-CoV-2 infection : Older age†	0.37	0.23	0.10	0.002
Male sex	-0.28	0·06	<0·001	0.014
Hypertension	-0.21	0·08	0.008	0·005

B.

	Beta*	SE	P	Partial r2
Prior SARS-CoV-2 infection	1.86	0.13	<0.001	0.115
Age	-0.01	0.00	<0.001	0.019
Male sex	-0.24	0.06	<0.001	0.010
Prior SARS-CoV-2 infection : Male sex	-0.42	0.25	0.08	0.002
Hypertension	-0.18	0.08	0.034	0.003

C.

	Beta*	SE	P	Partial r2
Prior SARS-CoV-2 infection	1.61	0.12	<0.001	0.105
Age	-0.01	0.00	<0.001	0.019
Male sex	-0.27	0.06	<0.001	0.013
Hypertension	-0.23	0.08	0.005	0.005
Prior SARS-CoV-2 infection : Hypertension	1.17	0.35	0.001	0.008

D.

	Beta*	SE	P	Partial r2
Prior SARS-CoV-2 infection	1.72	0.11	<0.001	0.133
Older Age†	-0.20	0.07	0.005	0.005
Male sex	-0.15	0.09	0.11	0.002
Older Age† x Male sex (*interaction term*)	-0.25	0.12	0.043	0.003
Hypertension	-0.22	0.08	0.007	0.005

*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable).

† Older age defined as age greater than the median age of the cohort (41.7 years).

Supplemental Table 4. Correlates of longitudinal anti-spike IgG antibody response following complete initial mRNA vaccination, stratified by prior SARS-CoV-2 infection status.

	No Prior SARS-CoV-2 Infection N=784			Prior SARS-CoV-2 Infection N=59		
	Beta*	SE	P	Beta*	SE	P
Age, year	-0·01	0·00	<0·001	-0·00	0·01	0·74
Male sex	-0·24	0·06	<0·001	-0·72	0·33	0·032
Hypertension	-0·23	0·08	0·005	0·96	0·50	0·06

*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable), in analyses adjusted for age, sex, and hypertension.

Supplemental Table 5. Association of prior SARS-CoV-2 infection with longitudinal anti-spike IgG antibody response following complete initial mRNA vaccination, stratified by age, sex, and hypertension status.

A.

	Age <42 years N=421			Age ≥42 years N=422		
	Beta*	SE	P	Beta*	SE	P
Prior SARS-CoV-2 infection	1·57	0·13	<0·001	1·93	0·19	<0·001

*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable), in analyses adjusted sex and hypertension.

B.

	Males N=256			Females N=587		
	Beta*	SE	P	Beta*	SE	P
Prior SARS-CoV-2 infection	1·35	0·20	<0·001	1·86	0·13	<0·001

*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable), in analyses adjusted for age and hypertension.

C.

	No Hypertension N=715			Hypertension N=128		
	Beta*	SE	P	Beta*	SE	P
Prior SARS-CoV-2 infection	1·61	0·11	<0·001	2·77	0·43	<0·001

*Beta values represent increase in 1-SD of log(10)IgG-S level per presence (vs absence) of a categorical variable or per unit increment of continuous variable), in analyses adjusted for age and sex.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	8

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*9*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*NA*
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14
Generalisability	21	Discuss the generalisability (external validity) of the study results	13-14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.