PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Ebinger, Joseph; Joung, Sandy; Liu, Yunxian; Wu, Min; Weber, Brittany; Claggett, Brian; Botting, Patrick; Sun, Nancy; Driver, Matthew; Kao, Yu Hung; Khuu, Briana; Wynter, Timothy; Nguyen, Trevor Trung; Alotaibi, Mona; Prostko, John; Frias, Edwin; Stewart, James; Goodridge, Helen; Chen, Peter; Jordan, Stanley; Jain, Mohit; Sharma, Sonia; Fert-Bober, Justyna; Van Eyk, Jennifer; Minissian, Margo; Arditi, Moshe; Melmed, Gil; Braun, Jonathan; McGovern, Dermot; Cheng, Susan; Sobhani, Kimia

VERSION 1 – REVIEW

REVIEWER	Cohen, Regev Laniado Hospital, Infectious disease
REVIEW RETURNED	25-Dec-2021

GENERAL COMMENTS	Ebinger et al report on a long term follow-up of humoral response to BNT162b2 vaccine among over 800 HCWs. They have shown long-lasting (10 months) anti IgG-S levels in most of the participants with several factors influencing the levels, including sex, age, history of COVID infection and hypertension. The manuscript is well written and the results are important. My comments: 1. The number of cases in the study cohort is stated as 828 cases (as appearing in the abstract and methods), although in the supp table 1 and in Fig 1, the number is different, being 843 cases. As a matter of fact, when calculation in made from the data provided by the authors, in the Methods, the conclusion is that the number 828 is probably wrong. If this is only a typo, it should be corrected, but if this mistake represent a deeper problem in the complicated statistical analysis done in this paper, the whole analysis should be reviewed. Similarly, the total number of cases in the text is 1703, while in the Supp table 1 is 1689. 2. Results are shown in the Methods section, and should better appear elsewhere. 3. The authors do not state the dates in which this study was conducted. 4. In Supp table 1 there is a comparison between included and excluded cases. I could not find the description of the 846 excluded cases in the methods or elsewhere. Who were these 846 cases? Are those the 860 excluded cases described in the methods? Please explain. 5. The Results section is disorganized in my opinion. It begins with reference to Supp. table 1. If this is important to start with, why

	assign it to be as a Supp? In the first paragraph of the results the authors refer to "all prior infected individuals", but these important subsets of cases, which also behaves differently and are discussed later on in the paper, are not mentioned in the results. In fact, only in Supp table 2 the reader may find out that there were 59 cases in this group (and Supp table 2 is not at all referred to in the text). I would suggest that the number of cases in the cohort, excluded cases and the subsets of cases will appear first in the Results section, and rechecked for accuracy. 6. I understand that the 59 cases were infected with wild type of SARS-CoV-2 before they received the vaccinations. Could the authors comment on what period of time elapsed between infection and vaccination? What probable variants infected those patients? Was the local policy at the time to suggest a full vaccination course (2 doses) for COVID-recovered HCWs? 7. Supp. figure 1 should be considered to be assigned as Figure 1. As Supp table 2 should be considered to be Table 2. 8. The fact that the cohort of cases in this study is composed of young population should be more stressed in the conclusions. 75% of the cases are younger than 53 years, hence when making conclusions regarding the findings of this study, it would better be stated that it relates to generally young population. The authors do relate to this point in the limitations. 9. Are there any data regarding the HCWs in terms of their potential of recurrent exposure to COVID patients? High risk wards, as COVID wards and ICUs, may expose these HCWs to repeated encounters with the wild virus and to boosting of the immune response. The authors may want to comment on this point. A minor comment: S/C (page 7 line 52) and AIC (page 8 line 17) should be explained.

REVIEWER	He, Qiushui University of Turku
REVIEW RETURNED	17-Jan-2022

GENERAL COMMENTS	This study was aimed to evaluate the demographic and clinical factors associated with variations in longitudinal antibody response following completion of 2-dose regiment of BNT162b2 vaccination. The study design and the methods used were proper. The number of study subjects was large, and the findings are important. The manuscript was well written. The following concern should be discussed. Since the plasma samples were collected within 7 to 21 days after dose 1 and dose 2, whether the timing differences in collection of samples could contribute to the variation and persistence of antibodies in different individuals should be discussed.

REVIEWER	Wong, Sook-San Guangzhou Medical University
REVIEW RETURNED	31-Jan-2022

GENERAL COMMENTS	The authors analyzed the longitudinal serological response in a cohort of healthcare workers after the receipt of the BNT162b2 mRNA vaccine. They used mixed linear models to determine the factors associated with robust post-vaccination antibody response. They found that prior SARS-CoV-2 infection is the best indicator of sustained and elevated antibody response, as well as the

	contribution of hypertension and female sex to the response. In general, the manuscript is concise and specific about it's aims. My main comments is that the MS can benefit from further explanation or details. Specifically: 1. First paragraph of the Results section- is it supposed to be Supplemental Table 2 that should be cited? Supplemental Table 1 seems to be something else and not discussed in the main text. 2. Results section, line 34- what were the breakdown of sexes in those <42 vs >42 years old? Were there any interactions? 3. Figures- the start of the log[IgG] spline (at -4 weeks) for those with prior infection appears to be same as those without prior infection. This appears to be misleading, both from a biological and data standpoint, as the majority of the prior infected samples had antibody titers above the threshold. Can this be corrected? 4. Flow diagram- what does the excluded Non BNT162b2-exposed (n=23) group refer to? 5. Please indicate the number of data available for each group at each time point for the respective analyses/figure. Please update the STROBE checklist where appropriate. 6. Page 16, Line 8- "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." Which general population does the author want to compare to? A general population would include children, for example. Consider deleting. 7. Suggest clarifying that the vaccine does not contain N-protein, hence IgG[N] positivity is suggestive of prior infection. 8. Along this line- any indication of what window period were the prior exposures? Any indication with the anti-N IgG response? 9. Abstract: the total cohort number is 823, does not match with main text. 10. Introduction: Line 10, infectious disease should be replaced with infections or disease. 11. Other minor language corrections- see attached file.

VERSION 1 – AUTHOR RESPONSE

Comments from Reviewer #1
Dr. Regev Cohen, Laniado Hospital

Ebinger et al report on a long term follow-up of humoral response to BNT162b2 vaccine among over 800 HCWs. They have shown long-lasting (10 months) anti IgG-S levels in most of the participants with several factors influencing the levels, including sex, age, history of COVID infection and hypertension. The manuscript is well written and the results are important.

Reply: We thank the Reviewer for the valuable comments and suggestions provided.

Comments:

1. The number of cases in the study cohort is stated as 828 cases (as appearing in the abstract and methods), although in the supp table 1 and in Fig 1, the number is different, being 843 cases. As a matter of fact, when calculation in made from the data provided by the authors, in the Methods, the conclusion is that the number 828 is probably wrong. If this is only a typo, it

should be corrected, but if this mistake represent a deeper problem in the complicated statistical analysis done in this paper, the whole analysis should be reviewed. Similarly, the total number of cases in the text is 1703, while in the Supp table 1 is 1689.

Reply: We very much thank the Reviewer for identifying the typographical error in the Abstract, and this has now been corrected to read 843. Regarding the values displayed in Supplemental Table 1, we recognize that we may have created confusion because this presentation of the data excludes the 14 individuals without available medical history. We omitted these individuals from these analyses due to their missing data precluding comparisons of obesity, hypertension and the Charlson comorbidity index. We agree with the Reviewer that this was both unclear and not well explained. As such, we have updated Supplemental Table 1 to include these 14 individuals, along with a footnote clarifying that data were not available for a small subset of this cohort for the variables specified.

Page 3, Paragraph 4: "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."

Page 9, Paragraph 1: "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."

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.

1. Results are shown in the Methods section, and should better appear elsewhere.

Reply: We thank the Review for identifying this issue and have now moved all results from the Methods section to the Results section, as helpfully suggested:

Page 9, Paragraph 1: "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."

1. The authors do not state the dates in which this study was conducted.

Reply: We agree with the Reviewer that study dates should be included and have now added these important details to the revised manuscript:

Page 9, Paragraph 1: "A total of 1,703 healthcare workers were enrolled in the source cohort between November 30, 2020 and November 11, 2021."

1. In Supp table 1 there is a comparison between included and excluded cases. I could not find the description of the 846 excluded cases in the methods or elsewhere. Who were these 846 cases? Are those the 860 excluded cases described in the methods? Please explain.

Reply: We thank the Reviewer for astutely identifying how greater clarification is needed for presenting and describing the data shown in this table and elsewhere. As the Reviewer correctly surmised, these are indeed the n=860 excluded cases and we have updated the Methods section text to clarify the total exclusions (below). We have also added further clarification to the footnote of the revised Supplemental Table 1 to clarify that the n=846 are excluded individuals who did not have missing medical history data available for clinical characteristics comparisons shown in Supplemental Table 1. We appreciate the opportunity to clarify these important details pertaining to the sampling strategy.

Page 9, Paragraph 1: "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)."

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.

1. The Results section is disorganized in my opinion. It begins with reference to Supp. table 1. If this is important to start with, why assign it to be as a Supp? In the first paragraph of the results the authors refer to "all prior infected individuals", but these important subsets of cases, which also behaves differently and are discussed later on in the paper, are not mentioned in the results. In fact, only in Supp table 2 the reader may find out that there were 59 cases in this group (and Supp table 2 is not at all referred to in the text). I would suggest that the number of cases in the cohort, excluded cases and the subsets of cases will appear first in the Results section, and rechecked for accuracy.

Reply: We agree with the reviewer that the results section would benefit from reorganization, particularly focused on the first paragraph. As recommended, we have moved the cohort development data to this section and moved Supplemental Figure 1 and Supplemental Table 2 to the main document as Figure 1 and Table 1. We have also reordered the section to ensure a more chronologically ordered progression of information around study participants with a history of prior SARS-CoV-2 infection.

Page 9, Paragraph 1: "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."

1. I understand that the 59 cases were infected with wild type of SARS-CoV-2 before they received the vaccinations. Could the authors comment on what period of time elapsed between infection and vaccination? What probable variants infected those patients? Was the local policy at the time to suggest a full vaccination course (2 doses) for COVID-recovered HCWs?

Reply: The Reviewer raises an important point regarding the timing from prior SARS-CoV-2 infection to vaccination. Because some participants received COVID-19 testing outside of our health system, or underwent IgG-N testing, the exact date of infection is not available for all 59 participants. For those with an available date (n=28), the average time from infection to first vaccine dose was 139 days. We have now included this information in the revised manuscript, as shown below. Because viral variant testing was not completed on participant samples, we unfortunately cannot confirm which variants contributed to infection in each participant. According to local department of health and institutional policies, all healthcare workers were advised to receive 2 doses of mRNA vaccine. We have now added discussion of these important details to the revised manuscript:

Page 9, Paragraph 1: "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)."

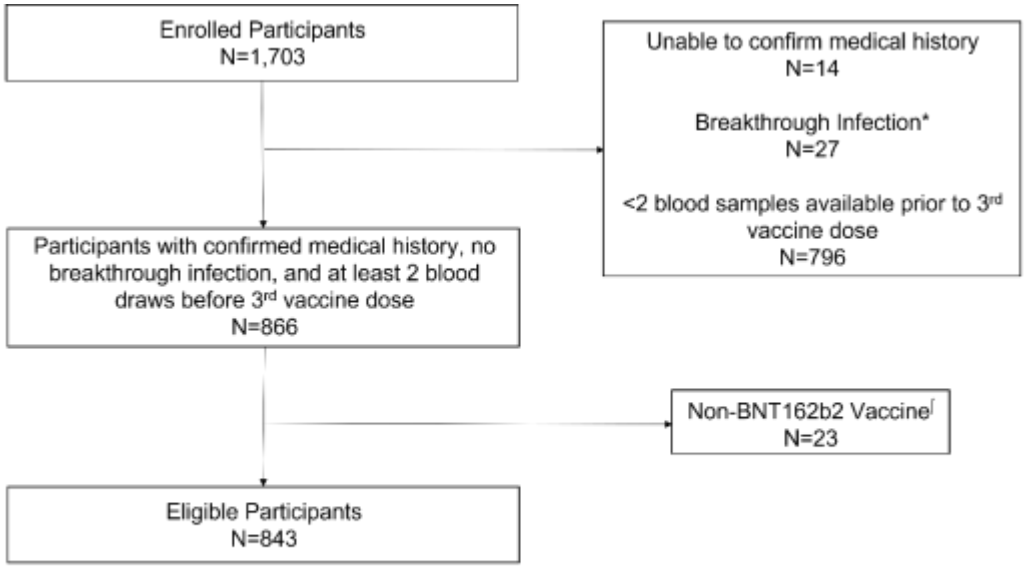
Page 15, Paragraph 1: "Because viral variant testing was not routinely conducted for participant samples, data on which variants contributed to confirmed infections were not available for analyses."

Page 7, Paragraph 1: "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."

1. Supp. figure 1 should be considered to be assigned as Figure 1. As Supp table 2 should be considered to be Table 2.

Reply: We completely agree with the Reviewer that both these supplemental data elements are important and merit inclusion in the main body of the manuscript. As such, we have moved them to from the supplement to the main manuscript body as helpfully suggested:

Figure 1: Cohort Development Flow Diagram.



*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.

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.

1. The fact that the cohort of cases in this study is composed of young population should be more stressed in the conclusions. 75% of the cases are younger than 53 years, hence when making conclusions regarding the findings of this study, it would better be stated that it relates to generally young population. The authors do relate to this point in the limitations.

Reply: We completely agree with the Reviewer on this important point. We have now included additional emphasis on the younger age range of our cohort as helpfully suggested:

Page 13, Paragraph 2: “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.”

1. Are there any data regarding the HCWs in terms of their potential of recurrent exposure to COVID patients? High risk wards, as COVID wards and ICUs, may expose these HCWs to repeated encounters with the wild virus and to boosting of the immune response. The authors may want to comment on this point.

Reply: The Reviewer raises an important point regarding risk of recurrent COVID-19 exposures among healthcare workers. As helpfully suggested, we have now included this information in our Results section, as well as in Table 1. We have also added discussion regarding this important point to our limitations section.

Page 9, Paragraph 1: “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).”

Page 14, Paragraph 3: “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.”

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
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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
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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.

Minor comment:

1. S/C (page 7 line 52) and AIC (page 8 line 17) should be explained.

Reply: We thank the Reviewer for identifying the need to clarify these terms. As helpfully suggested, we have now included clarification that S/C as the signal to cutoff index and we also provide a more detailed explanation of our use of Akaike Information Criterion (AIC).

Page 7, Paragraph 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]”

Page 8, Paragraph 2: “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.”

Comments from Reviewer #2
Dr. Qiushui He, University of Turku

This study was aimed to evaluate the demographic and clinical factors associated with variations in longitudinal antibody response following completion of 2-dose regiment of BNT162b2 vaccination. The study design and the methods used were proper. The number of study subjects was large, and the findings are important. The manuscript was well written.

Reply: We thank the Reviewer for the valuable comments and suggestions provided.

1. The following concern should be discussed. Since the plasma samples were collected within 7 to 21 days after dose 1 and dose 2, whether the timing differences in collection of samples

could contribute to the variation and persistence of antibodies in different individuals should be discussed.

Reply: We thank the Reviewer for this astute query regarding possible effects arising from potential time-dependent variation within the initial sampling windows. We completely agree that this issue should be highlighted and so we have now added discussion of this important point to the revised manuscript, as helpfully suggested:

Page 15, Paragraph 1: “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.”

Comments from Reviewer #3
Dr. Sook-San Wong, Guangzhou Medical University

The authors analyzed the longitudinal serological response in a cohort of healthcare workers after the receipt of the BNT162b2 mRNA vaccine. They used mixed linear models to determine the factors associated with robust post-vaccination antibody response. They found that prior SARS-CoV-2 infection is the best indicator of sustained and elevated antibody response, as well as the contribution of hypertension and female sex to the response. In general, the manuscript is concise and specific about it's aims. My main comments is that the MS can benefit from further explanation or details.

Reply: We thank the Reviewer for the valuable comments and suggestions provided.

1. First paragraph of the Results section- is it supposed to be Supplemental Table 2 that should be cited? Supplemental Table 1 seems to be something else and not discussed in the main text.

Reply: We thank the Reviewer for identifying the typographical error that led to reversed labeling of these supplemental tables. We have now moved Supplemental Table 2 into the main body of the manuscript text (now Table 1) and corrected the labeling. As helpfully suggested, we have also clarified references to the supplemental tables in the main text.

Page 9, Paragraph 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.”

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.

1. Results section, line 34- what were the breakdown of sexes in those <42 vs >42 years old? Were there any interactions?

Reply: We thank the Reviewer for this astute and important query. We have now included additional details regarding the breakdown of sex and other characteristics by younger (<42 years) vs older (>42 years) age in Supplemental Table 2. As shown below, the proportion of males was lower in the younger aged participants (25%) compared to the in the older aged participants (36%). As helpfully suggested, we have now conducted analyses assessing for age*sex interaction and this was found to be significant with results showing that older age (above the median cohort age of 42 years) was associated with even lower antibody levels among males compared to females. We have now added report of these important analyses to the revised manuscript:

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.

Page 10, Paragraph 2: “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).”

Supplemental Table 3D: Clinical and demographic correlates of longitudinal anti-spike IgG antibody response following complete initial mRNA vaccination, including and interaction term for age and sex.

	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).

1. Figures- the start of the log[IgG] spline (at -4 weeks) for those with prior infection appears to be same as those without prior infection. This appears to be misleading, both from a biological and data standpoint, as the majority of the prior infected samples had antibody titers above the threshold. Can this be corrected?

Reply: We appreciate this important observation and comment from the Reviewer. We have now closely re-examined all data and analyses, and we can confirm that the data collected at baseline (up to approximately 4 weeks prior to receiving the second dose of vaccine) are correct even for those individuals who had prior infection. We ascertained that the relatively lower antibody levels for these individuals is due to what has been previously reported as expected antibody decay within the 4 to 6 month period following natural SARS-CoV-2 infection (e.g. *Cohen et al, Cell Reports Medicine 2021:2:100354*). For those participants in whom dates of prior infection were available, we find that the average number of days between prior infection and first vaccine dose was 139 days, with a range of 14 to 292 days. Thus, given that a majority of these participants had their infection at least 4 months and up to 10 months prior to vaccination, the baseline antibody levels seen our study are noted to be relatively consistent with observed or predicted values from other studies. We have now added details regarding these important points to the revised manuscript:

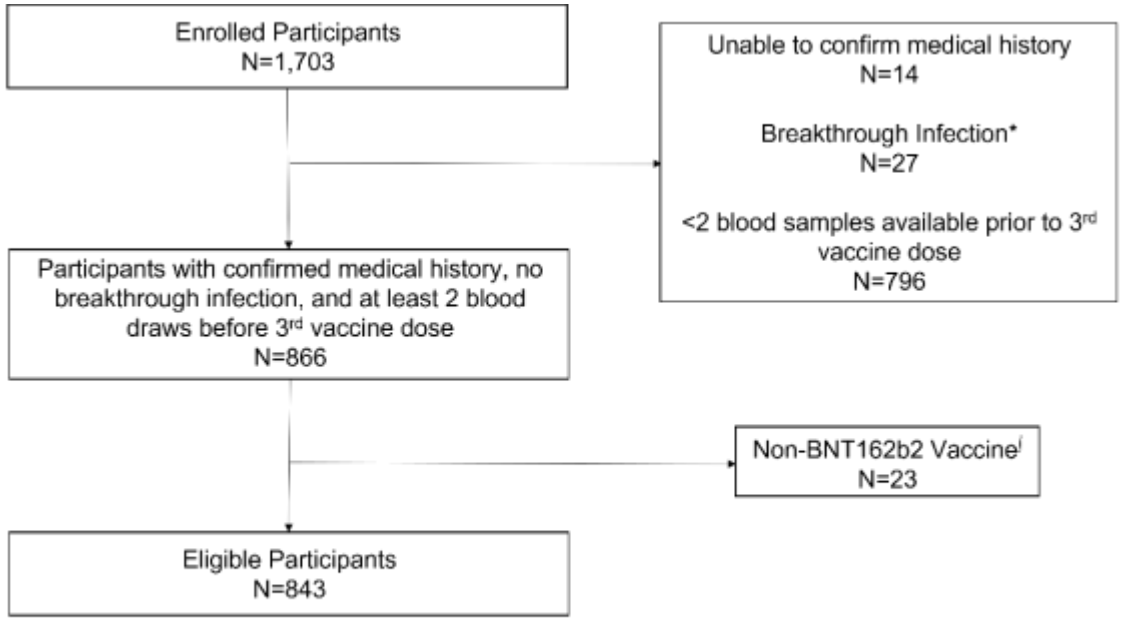
Page 9, Paragraph 1: “Among participants for whom the date of first positive SARS-CoV-2 PCR was available (n=28), the average time from prior infection to first vaccine dose was 139 days (range 14-292 days).”

Page 15, Paragraph 1: “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.”

1. Flow diagram- what does the excluded Non BNT162b2-exposed (n=23) group refer to?

Reply: We thank the Reviewer for identifying an important area in need of clarification. This N=23 subset includes those individuals who received vaccine platforms other than BNT162b2. These individuals were excluded from analyses to avoid heterogeneity of results when analyzing in the longitudinal immune response, particularly given the relatively small number of these individuals in our source cohort. We have now revised the figure to provide more clarification including a footnote that specifies these individuals did not receive BNT162b2 vaccine:

Figure 1: Cohort Development Flow Diagram.



*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.

[j] Participants who received any vaccine other than BNT162b2.

1. Please indicate the number of data available for each group at each time point for the respective analyses/figure.

Reply: We appreciate this suggestion from the Reviewer and agree that including additional details on the number of available samples contributing data at each time point would be helpful for interpreting the results of analyses as displayed in the spline curves. Therefore, we have now added Supplemental Figures 1 A-D, which display the number of samples available at each time point while stratified by prior COVID-19 status, age, sex and hypertension. An example of the raw count data is also provided, below for reference:

Weeks from Vaccine Dose 2	No Prior SARS-CoV-2 Infection	Prior SARS-CoV-2 Infection
(-5,-4]	3	1
(-4,-3]	398	28
(-3,-2]	151	9
(-2,-1]	34	2
(-1,0]	442	27
(0,1]	59	4
(1,2]	62	6
(2,3]	190	11

(3,4]	69	2
(4,5]	22	0
(5,6]	30	6
(6,7]	212	16
(7,8]	222	15
(8,9]	111	8
(9,10]	30	1
(10,11]	13	0
(11,12]	15	0
(12,13]	12	1
(13,14]	9	1
(14,15]	66	8
(15,16]	109	10
(16,17]	158	6
(17,18]	131	8
(18,19]	64	3
(19,20]	33	4
(20,21]	57	6
(21,22]	93	12
(22,23]	75	4
(23,24]	83	5
(24,25]	87	2
(25,26]	47	7
(26,27]	79	5
(27,28]	66	5
(28,29]	28	1
(29,30]	15	3
(30,31]	25	0
(31,32]	58	3
(32,33]	155	12
(33,34]	106	8
(34,35]	84	5
(35,36]	78	3
(36,37]	50	3
(37,38]	37	3
(38,39]	27	1
(39,40]	4	1

1. Please update the STROBE checklist where appropriate.

Reply: We thank the Reviewer for reminding us to update the STROBE checklist following our edits and based on the helpful input from all Reviewers. We have now included the updated checklist along with our revised manuscript.

1. Page 16, Line 8- "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." Which general population does the author want to compare to? A general population would include children, for example. Consider deleting.

Reply: We completely agree with the Reviewer on this important point and had intended to specify comparison to the general adult population. To avoid any confusion, we have deleted this statement as helpfully suggested.

1. Suggest clarifying that the vaccine does not contain N-protein, hence IgG[N] positivity is suggestive of prior infection.

Reply: We completely agree with this helpful suggestion from the Reviewer. We have now added clarification regarding the contextual significance of IgG to nucleocapsid measures, especially when detected at elevated levels:

Page 7, Paragraph 1: “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.”

1. Along this line- any indication of what window period were the prior exposures? Any indication with the anti-N IgG response?

Reply: The Reviewer raises an important point regarding the timing from prior SARS-CoV-2 infection to vaccination. Because some participants received COVID-19 testing outside of our health system, or underwent IgG-N testing only (without prior viral PCR testing data available), the exact date of infection is not available for all 59 participants. For those with data available including date information (n=28), the average time from infection to first vaccine dose was 139 days. We also observed that the IgG-N testing data acquired prior to first vaccination revealed values that were generally not very elevated, even in persons who reported prior infection and/or had prior PCR testing data available to confirm prior infection. We interpret these findings as consistent with the known temporality of antibody (i.e. IgG-N) decay following natural infection when their antibody levels are measured up to several months after recovery. We have now included this information in the revised manuscript:

Page 9, Paragraph 1: “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).”

Page 15, Paragraph 1: “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.”

1. Abstract: the total cohort number is 823, does not match with main text.

Reply: We very much thank the Reviewer for identifying the typographical error in the Abstract, and this has now been corrected to read 843.

Page 3, Paragraph 4: “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.”

1. Introduction: Line 10, infectious disease should be replaced with infections or disease.

Reply: We thank the Reviewer for identifying this issue. We have now made corrections to the text, as helpfully suggested:

Page 6, Paragraph 1: “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. Other minor language corrections- see attached file. [SEE ATTACHED FILE FOR STICKY NOTE COMMENTS FROM THIS REVIEWER ON THE MANUSCRIPT PDF]

Reply: We are grateful to the Reviewer for taking the time to very helpfully annotate the PDF document. We have revised the manuscript to include all of the corrections based on the excellent suggestions provided.
1

VERSION 2 – REVIEW

REVIEWER	Wong, Sook-San Guangzhou Medical University
REVIEW RETURNED	29-Mar-2022

GENERAL COMMENTS	All concerns were addressed well.