THE LANCET
Microbe

# Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: ChemaitellyH, Ayoub HH, AlMukdad S, et al. Protection from previous natural infection compared with mRNA vaccination against SARS-CoV-2 infection and severe COVID-19 in Qatar: a retrospective cohort study. *Lancet Microbe* 2022; published online November 11. https://doi.org/10.1016/S2666-5247(22)00287-7.

# Appendix

**Table of Contents**

## *Section S1:* Laboratory methods and variant ascertainment

### Real-time reverse-transcription polymerase chain reaction testing

Nasopharyngeal and/or oropharyngeal swabs were collected for polymerase chain reaction (PCR) testing and placed in Universal Transport Medium (UTM). Aliquots of UTM were: 1) extracted on KingFisher Flex (Thermo Fisher Scientific, USA), MGISP-960 (MGI, China), or ExiPrep 96 Lite (Bioneer, South Korea) followed by testing with real-time reverse-transcription PCR (RT-qPCR) using TaqPath COVID-19 Combo Kits (Thermo Fisher Scientific, USA) on an ABI 7500 FAST (Thermo Fisher Scientific, USA); 2) tested directly on the Cepheid GeneXpert system using the Xpert Xpress SARS-CoV-2 (Cepheid, USA); or 3) loaded directly into a Roche cobas 6800 system and assayed with the cobas SARS-CoV-2 Test (Roche, Switzerland). The first assay targets the viral S, N, and ORF1ab gene regions. The second targets the viral N and E-gene regions, and the third targets the ORF1ab and E-gene regions.

All PCR testing was conducted at the Hamad Medical Corporation Central Laboratory or Sidra Medicine Laboratory, following standardized protocols.

### Rapid antigen testing

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigen tests were performed on nasopharyngeal swabs using one of the following lateral flow antigen tests: Panbio COVID-19 Ag Rapid Test Device (Abbott, USA); SARS-CoV-2 Rapid Antigen Test (Roche, Switzerland); Standard Q COVID-19 Antigen Test (SD Biosensor, Korea); or CareStart COVID-19 Antigen Test (Access Bio, USA). All antigen tests were performed point-of-care according to each manufacturer's instructions at public or private hospitals and clinics throughout Qatar with prior authorization and training by the Ministry of Public Health (MOPH). Antigen test results

were electronically reported to the MOPH in real time using the Antigen Test Management System which is integrated with the national Coronavirus Disease 2019 (COVID-19) database.

**Classification of infections by variant type**

Surveillance for SARS-CoV-2 variants in Qatar is based on viral genome sequencing and multiplex RT-qPCR variant screening[1] of random positive clinical samples,[2-7] complemented by deep sequencing of wastewater samples.[4,8] Further details on the viral genome sequencing and multiplex RT-qPCR variant screening throughout the SARS-CoV-2 waves in Qatar can be found in previous publications.[2-7,9-12]

## *Section S2:* COVID-19 severity, criticality, and fatality classification

Severe COVID-19 disease was defined per the World Health Organization (WHO) classification as a SARS-CoV-2 infected person with "oxygen saturation of <90% on room air, and/or respiratory rate of >30 breaths/minute in adults and children >5 years old (or ≥60 breaths/minute in children <2 months old or ≥50 breaths/minute in children 2-11 months old or ≥40 breaths/minute in children 1–5 years old), and/or signs of severe respiratory distress (accessory muscle use and inability to complete full sentences, and, in children, very severe chest wall indrawing, grunting, central cyanosis, or presence of any other general danger signs)".[13] Detailed WHO criteria for classifying SARS-CoV-2 infection severity can be found in the WHO technical report.[13]

Critical COVID-19 disease was defined per WHO classification as a SARS-CoV-2 infected person with "acute respiratory distress syndrome, sepsis, septic shock, or other conditions that would normally require the provision of life sustaining therapies such as mechanical ventilation (invasive or non-invasive) or vasopressor therapy".[13] Detailed WHO criteria for classifying SARS-CoV-2 infection criticality can be found in the WHO technical report.[13]

COVID-19 death was defined per WHO classification as "a death resulting from a clinically compatible illness, in a probable or confirmed COVID-19 case, unless there is a clear alternative cause of death that cannot be related to COVID-19 disease (e.g. trauma). There should be no period of complete recovery from COVID-19 between illness and death. A death due to COVID-19 may not be attributed to another disease (e.g. cancer) and should be counted independently of preexisting conditions that are suspected of triggering a severe course of COVID-19". Detailed WHO criteria for classifying COVID-19 death can be found in the WHO technical report.[14]

## *Table S1:* Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist for cohort studies.

| | Item No | Recommendation | Main Text page |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | Abstract |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | Abstract |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | Introduction |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | Introduction |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | Methods ('Study design and study cohorts', 'Study inclusion criteria', 'Cohort matching and follow-up', & 'Study Outcomes') |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | Methods ('Study design and study cohorts', 'Study inclusion criteria', & 'Cohort matching and follow-up') & Figure 1 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | Methods ('Study inclusion criteria', & 'Cohort matching and follow-up') & Figure 1 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | Methods ('Study design and study cohorts', 'Study inclusion criteria', 'Cohort matching and follow-up', & 'Study Outcomes'), Table 1, & Sections S1 & S2 in Appendix |
| 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 | Methods ('Study population and data sources' & 'Statistical analysis', paragraph 1), Table 1, & Sections S1 & S2 in Appendix |
| Bias | 9 | Describe any efforts to address potential sources of bias | Methods ('Study inclusion criteria', 'Cohort matching and follow-up', & 'Statistical analysis', paragraph 2) |
| Study size | 10 | Explain how the study size was arrived at | Figure 1 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | Methods ('Cohort matching and follow-up') & Table 1 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | Methods ('Statistical analysis') |
| | | (*b*) Describe any methods used to examine subgroups and interactions | Methods ('Statistical analysis', paragraph 3) |
| | | (*c*) Explain how missing data were addressed | NA, see Methods ('Study population and data sources', paragraph 1) |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | NA, see Methods ('Study design and study cohorts', paragraph 1) |
| | | (*e*) Describe any sensitivity analyses | Methods ('Statistical analysis', paragraph 3) |
| **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 | Results ('Study population'), Figure 1, & Table 1 |
| | | (b) Give reasons for non-participation at each stage | |
| | | (c) Consider use of a flow diagram | |
| Descriptive data | 14 | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | Results ('Study population'), Table 1, & Figures S1 & S2 in Appendix |
| | | (b) Indicate number of participants with missing data for each variable of interest | Not applicable, see Methods ('Study population and data sources', paragraph 1) |
| | | (c) Summarise follow-up time (eg, average and total amount) | Results ('Natural infection versus BNT162b2 vaccination; Main |

| | | | |
|---|---|---|---|
| | | | analysis', paragraph 1 & 'Natural infection versus mRNA-1273 vaccination; Main analysis', paragraph 1), Figure 2, & Table 2 |
| Outcome data | 15 | Report numbers of outcome events or summary measures over time | Results ('Natural infection versus BNT162b2 vaccination; Main analysis' & 'Natural infection versus mRNA-1273 vaccination; Main analysis'), Figures 1-2, & Table 2 |
| 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 | Results ('Natural infection versus BNT162b2 vaccination; Main analysis', paragraphs 2-4 & 'Natural infection versus mRNA-1273 vaccination; Main analysis', paragraphs 2-4), Figure 2, & Table 2 |
| | | (b) Report category boundaries when continuous variables were categorized | Table 1 |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | Not applicable |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | Results ('Natural infection versus BNT162b2 vaccination; Sensitivity analyses' & 'Natural infection versus mRNA-1273 vaccination; Sensitivity analyses'), & Table 3 |
| Discussion | | | |
| Key results | 18 | Summarise key results with reference to study objectives | Discussion, paragraphs 1-4 |
| 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 | Discussion, paragraphs 5-9 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | Discussion, paragraph 10 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | Discussion, paragraphs 5-9 |
| 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 | Acknowledgements |

***Figure S1:*** **Distribution of documented SARS-CoV-2 primary infections and of first-dose vaccinations by calendar month in the matched cohorts of the natural-infection-versus-BNT162b2-vaccination study (panels A and B) and the natural-infection-versus-mRNA-1273-vaccination study (panels C and D).**

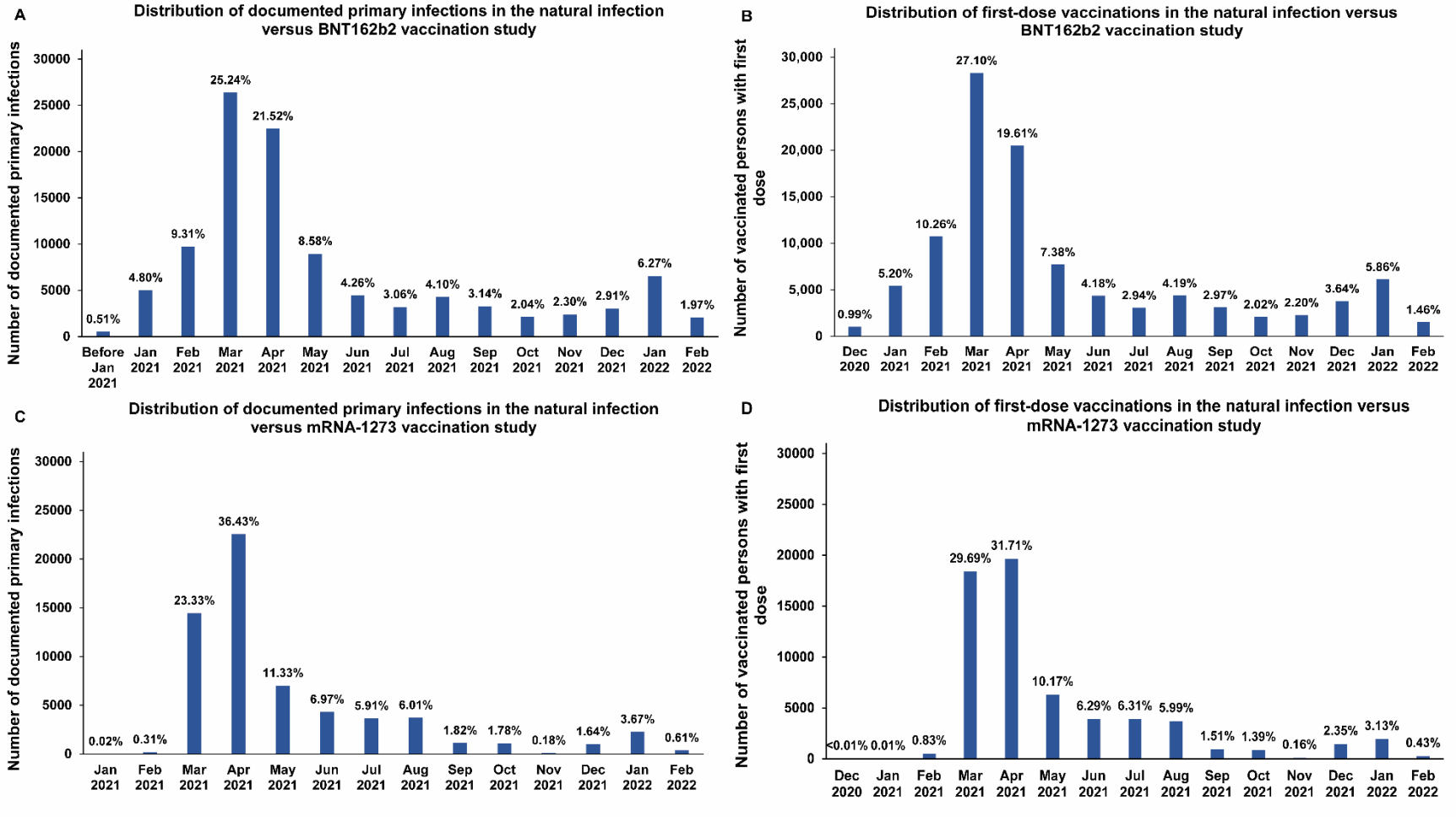

***Figure S2:*** **Distribution of the durations of follow-up in the matched cohorts of the A) natural-infection-versus-BNT162b2-vaccination study and B) natural-infection-versus-mRNA-1273-vaccination study. The increases and decreases in follow-up time frequency reflect timing of arrival of vaccine shipments and expansion of vaccination eligibility over time to new and younger age cohorts.**

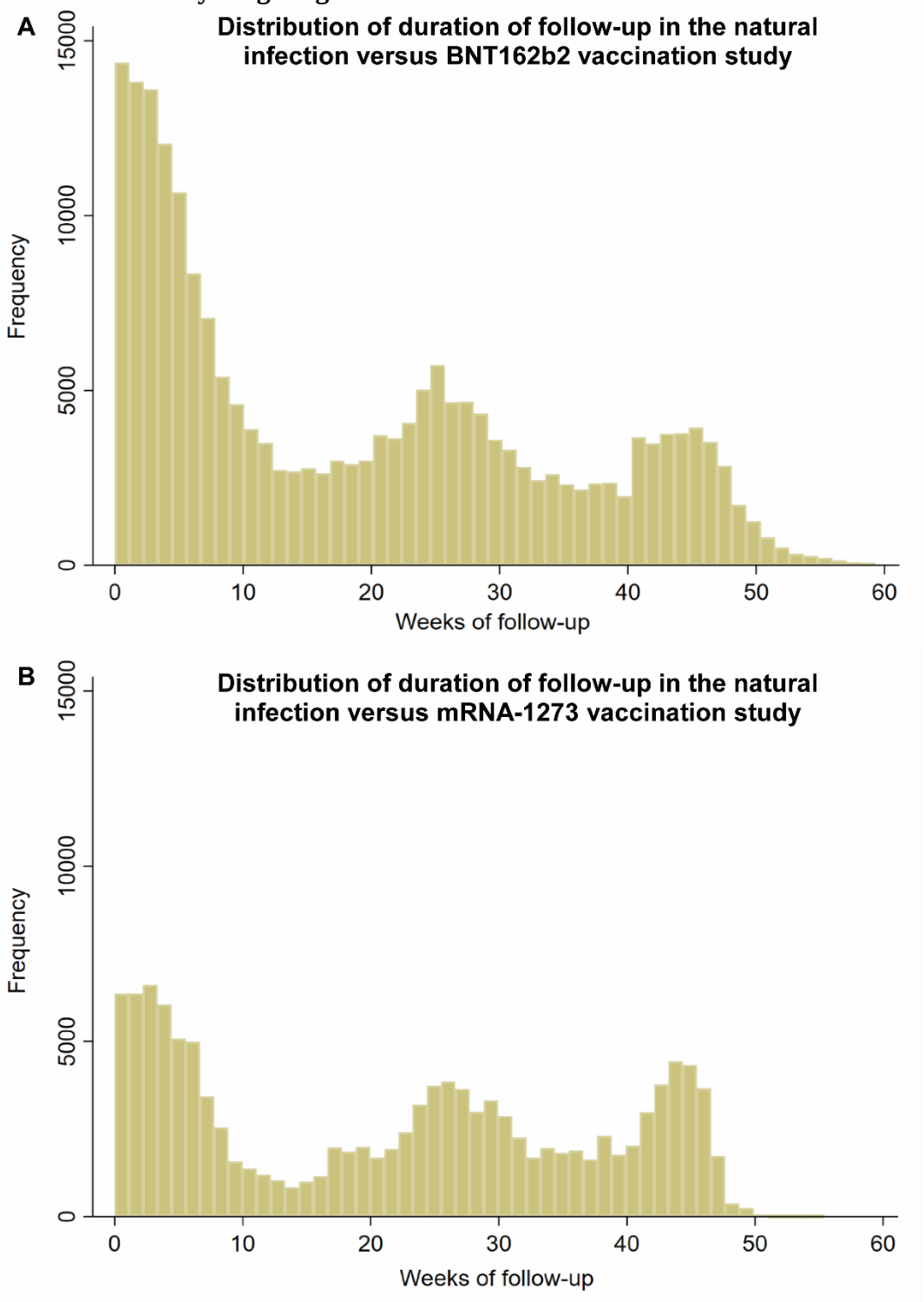

***Table S2:*** **Sensitivity analyses. Hazard ratios for the incidence of SARS-CoV-2 infection and incidence of severe, critical, or fatal COVID-19, comparing the natural-infection cohort to the BNT162b2- and mRNA-1273-vaccinated cohorts after A) restricting the analysis to those aged 50 years or older and B) stratifying the analysis by sex.**

| Epidemiological measure | Natural infection versus BNT162b2 vaccination study | | Natural infection versus mRNA-1273 vaccination study | |
|---|---|---|---|---|
| | Natural-infection cohort | BNT162b2-vaccinated cohort | Natural-infection cohort | mRNA-1273-vaccinated cohort |
| **A) Restricting the analysis to those aged 50 years or older** | | | | |
| Total follow-up time (person-weeks) | 136,140 | 132,914 | 90,306 | 88,538 |
| Incidence rate of infection (per 10,000 person-weeks) | 19.7 (17.5-22.2) | 35.7 (32.6-39.0) | 22.4 (19.5-25.7) | 33.6 (29.9-37.6) |
| Unadjusted hazard ratio for SARS-CoV-2 infection (95% CI) | 0.54 (0.46-0.63) | | 0.66 (0.55-0.79) | |
| Adjusted hazard ratio for SARS-CoV-2 infection* (95% CI) | 0.51 (0.44-0.60) | | 0.65 (0.54-0.78) | |
| Unadjusted hazard ratio for severe, critical, or fatal COVID-19† (95% CI) | 0.32 (0.10-1.00) | | 0.48 (0.09-2.64) | |
| Adjusted hazard ratio for severe, critical, or fatal COVID-19*† (95% CI) | 0.28 (0.09-0.89) | | 0.43 (0.08-2.43) | |
| **B) Stratifying the analysis by sex** | | | | |
| **Men** | | | | |
| Total follow-up time (person-weeks) | 1,337,778 | 1,338,942 | 984,612 | 962,626 |
| Incidence rate of infection (per 10,000 person-weeks) | 11.2 (10.7-11.8) | 27.6 (26.7-28.5) | 9.5 (8.9-10.1) | 22.7 (21.7-23.6) |
| Unadjusted hazard ratio for SARS-CoV-2 infection (95% CI) | 0.40 (0.38-0.42) | | 0.41 (0.38-0.44) | |
| Adjusted hazard ratio for SARS-CoV-2 infection* (95% CI) | 0.39 (0.37-0.42) | | 0.41 (0.38-0.44) | |
| Unadjusted hazard ratio for severe, critical, or fatal COVID-19† (95% CI) | 0.09 (0.01-0.68) | | 0.00 (0.00-2.37)‡ | |
| Adjusted hazard ratio for severe, critical, or fatal COVID-19*† (95% CI) | 0.08 (0.01-0.63) | | 0.00 (0.00-2.37)‡ | |
| **Women** | | | | |
| Total follow-up time (person-weeks) | 607,465 | 582,597 | 389,609 | 376,023 |
| Incidence rate of infection (per 10,000 person-weeks) | 33.6 (32.1-35.1) | 58.9 (57.0-61.0) | 35.2 (33.4-37.1) | 55.9 (53.5-58.3) |
| Unadjusted hazard ratio for SARS-CoV-2 infection (95% CI) | 0.55 (0.52-0.58) | | 0.55 (0.52-0.58) | |
| Adjusted hazard ratio for SARS-CoV-2 infection* (95% CI) | 0.54 (0.51-0.57) | | 0.61 (0.57-0.66) | |
| Unadjusted hazard ratio for severe, critical, or fatal COVID-19† (95% CI) | 0.69 (0.15-3.07) | | 0.48 (0.09-2.61) | |
| Adjusted hazard ratio for severe, critical, or fatal COVID-19*† (95% CI) | 0.77 (0.15-3.83) | | 0.40 (0.07-2.27) | |

CI denotes confidence interval, COVID-19 coronavirus disease 2019, and SARS-CoV-2 severe acute respiratory syndrome coronavirus 2.

*Cox regression analysis adjusted for sex, 10-year age group (Table 1), 10 nationality groups (Table 1), comorbidity count (Table 1), and timing of primary infection/first dose vaccination.

†Severity,[35] criticality,[35] and fatality[36] were defined according to the World Health Organization guidelines.

‡Estimate could not be generated using cox regression because of zero events in those with prior infection and therefore the hazard ratio was approximated by the incidence rate ratio.

## References


1. Multiplexed RT-qPCR to screen for SARS-COV-2 B.1.1.7, B.1.351, and P.1 variants of concern V.3. dx.doi.org/10.17504/protocols.io.br9vm966. 2021. (Accessed June 6, 2021, at https://www.protocols.io/view/multiplexed-rt-qpcr-to-screen-for-sars-cov-2-b-1-1-br9vm966.)
2. Abu-Raddad LJ, Chemaitelly H, Butt AA, National Study Group for Covid Vaccination. Effectiveness of the BNT162b2 Covid-19 Vaccine against the B.1.1.7 and B.1.351 Variants. N Engl J Med 2021;385:187-9.
3. Chemaitelly H, Yassine HM, Benslimane FM, et al. mRNA-1273 COVID-19 vaccine effectiveness against the B.1.1.7 and B.1.351 variants and severe COVID-19 disease in Qatar. Nat Med 2021;27:1614-21.
4. Qatar viral genome sequencing data. Data on randomly collected samples. https://www.gisaid.org/phylodynamics/global/nextstrain/. 2021. at https://www.gisaid.org/phylodynamics/global/nextstrain/.)
5. Benslimane FM, Al Khatib HA, Al-Jamal O, et al. One Year of SARS-CoV-2: Genomic Characterization of COVID-19 Outbreak in Qatar. Front Cell Infect Microbiol 2021;11:768883.
6. Hasan MR, Kalikiri MKR, Mirza F, et al. Real-Time SARS-CoV-2 Genotyping by High-Throughput Multiplex PCR Reveals the Epidemiology of the Variants of Concern in Qatar. Int J Infect Dis 2021;112:52-4.
7. Chemaitelly H, Tang P, Hasan MR, et al. Waning of BNT162b2 Vaccine Protection against SARS-CoV-2 Infection in Qatar. N Engl J Med 2021;385:e83.
8. Saththasivam J, El-Malah SS, Gomez TA, et al. COVID-19 (SARS-CoV-2) outbreak monitoring using wastewater-based epidemiology in Qatar. Sci Total Environ 2021;774:145608.
9. Altarawneh H, Chemaitelly H, Tang P, et al. Protection afforded by prior infection against SARS-CoV-2 reinfection with the Omicron variant. medRxiv In press at New England Journal of Medicine 2022:2022.01.05.22268782.
10. Chemaitelly H, Ayoub H, AlMukdad S, et al. Duration of mRNA vaccine protection against SARS-CoV-2 Omicron BA.1 and BA.2 subvariants in Qatar. medRxiv 2022:2022.03.13.22272308.
11. Abu-Raddad LJ, Chemaitelly H, Ayoub HH, et al. Effect of mRNA Vaccine Boosters against SARS-CoV-2 Omicron Infection in Qatar. N Engl J Med 2022.
12. Tang P, Hasan MR, Chemaitelly H, et al. BNT162b2 and mRNA-1273 COVID-19 vaccine effectiveness against the SARS-CoV-2 Delta variant in Qatar. Nat Med 2021;27:2136-43.
13. World Health Organization. COVID-19 clinical management: living guidance. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-1. Accessed on: May 31, 2021. 2021.
14. World Health Organization. International guidelines for certification and classification (coding) of COVID-19 as cause of death. Available from: https://www.who.int/classifications/icd/Guidelines_Cause_of_Death_COVID-19-20200420-EN.pdf?ua=1. Document Number: WHO/HQ/DDI/DNA/CAT. Accessed on May 31, 2021. 2021.