1-2023

Genetic Sequencing of Breakthrough Severe Acute Respiratory Syndrome Coronavirus 2 Infections in Fully Vaccinated Healthcare Workers

Sara Haddad

John Zurlo

Zi-Xuan Wang

Bonnie Richards

Nitika Badjatia

See next page for additional authors

Authors
Sara Haddad, John Zurlo, Zi-Xuan Wang, Bonnie Richards, Nitika Badjatia, and Dagan Coppock

OPEN

Genetic Sequencing of Breakthrough Severe Acute Respiratory Syndrome Coronavirus 2 Infections in Fully Vaccinated Healthcare Workers

Sara Haddad, MD, John Zurlo, MD,* Zi-Xuan Wang, PhD,† Bonnie Richards, DO,‡ Nitika Badjatia, PhD,† and Dagan Coppock, MD, MSCE**

Abstract: Introduction: Since the identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), several efforts have been made to prevent infection, culminating in the development of highly effective vaccines. However, as genetic variants of the virus have emerged, concerns have arisen regarding potential limitations of available vaccines.

Methods: In this article, we evaluated SARS-CoV-2 breakthrough infections among 520 healthcare workers in a 950-bed hospital from March 1, 2021, to October 31, 2021, and stratified them according to the type of vaccine received. We tested for associations between Alpha and Delta strains of SARS-CoV-2 and clinical characteristics.

Results: During the study period, only 0.85% of the patients with breakthrough infection vaccinated with messenger RNA (mRNA)-1273 required hospitalization, whereas none of patients vaccinated with BNT162b2 required admission. No association was noted between the variant isolated and the type of vaccination ($P = 0.474$). The cycle threshold was significantly lower for Delta strain infections compared with Alpha strain infections ($P = 0.0144$ for gene N1 and $P = 0.0271$ for gene N2), reflecting a significantly higher viral burden at the time of diagnosis of Delta strain infection. We observed that, at the time of diagnosis, almost all vaccinated patients had developed antispike antibodies, whereas slightly more than half had anti-N antibodies.

Discussion: We noted that vaccination remains an important preventive measure to protect patients from severe SARS-CoV-2 infection. The mRNA-1273 and BNT162b2 mRNA vaccines seem equally effective in minimizing the severity of breakthrough infections. Serologic patterns suggest that antispike antibodies may be more critical for protection than antinucleocapsid antibodies.

Key Words: SARS-CoV-2, variants of concern, cycle threshold, vaccination

(*Infect Dis Clin Pract* 2023;31: e1202)

Since the identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), several efforts have been made to prevent infection and disease, culminating in the development of highly effective vaccines.[1–5] However, as genetic variants of the virus have emerged, concerns have arisen regarding potential limitations of available vaccines.[6] The Centers for Disease Control and Prevention have classified these new variants based on specific properties such as transmissibility, associated morbidity and mortality, and the ability to evade natural immunity. For example, the variants B.1.1.7 (Alpha), B.1.351 (Beta), and P.1 (Gamma) have been labeled as variants being monitored, whereas the B.1.617.2 (Delta) and the B.1.1.529 (Omicron) variants have been labeled as variants of concern.[3,7–9]

Some variants such as the Alpha, Delta, and Omicron variants have been observed to be significantly more associated with breakthrough infection than other variants.[10–14] Breakthrough infection refers to viral acquisition in individuals who have been already fully vaccinated.[15] Although healthcare workers (HCWs) were among the first individuals to be vaccinated,[16] their ongoing exposure also placed them at risk for breakthrough infection.[17]

In this article, we describe outcomes of HCWs who experienced breakthrough SARS-CoV-2 infections as stratified by the type of vaccine received. We also test for associations between Alpha and Delta strains of SARS-CoV-2 and clinical characteristics.

METHODS

Subjects

The study was conducted at Thomas Jefferson University Hospital (TJUH) from March 1, 2021, to October 31, 2021. The study population included HCWs employed at TJUH during this period. Inclusion criteria included receipt of 2 doses of either the messenger RNA (mRNA) BNT162b2 (Pfizer-BioNTech) or the mRNA-1273 (Moderna) coronavirus disease 2019 (COVID-19) vaccine and documentation of subsequent COVID-19, that is, "vaccine breakthrough." Patients were excluded from the analysis if they received the Ad26.COV2.S (Janssen/Johnson & Johnson) COVID-19 vaccine. Clinical data were collected as a part of routine occupational health COVID-19 infection evaluations. A waiver of consent was provided by the Thomas Jefferson University Office of Human Research Institutional Review Board (Protocol No. 21E.441).

Vaccine Breakthrough Case Identification

Vaccine breakthrough cases were identified by screening employees who called into the Jefferson employee COVID-19 hotline. Employees were considered to have acquired breakthrough cases if they tested positive at least 2 weeks after receipt of their final vaccine dose in the series. Testing was performed using a nasopharyngeal swab reverse transcriptase-polymerase chain reaction test for SARS-CoV-2.

Data Sources

Clinical data were extracted from TJUH employee health records maintained on a PureOS-based database at the Jefferson Occupational Health Network. Gene sequencing data were collected from data maintained at the Thomas Jefferson University Molecular and Genomic Pathology Clinical Laboratory.

From the *Division of Infectious Diseases, †Department of Pathology, Anatomy and Cell Biology, and ‡Jefferson Occupational Health Network for Employees and Students, Thomas Jefferson University, Philadelphia, PA.

Correspondence to: Sara Haddad, MD, Jefferson Center, 1101 Market St, Ste 2720, Philadelphia, PA 19107. E-mail: Haddadsarah91@gmail.com.

The authors have no funding or conflicts of interest to disclose.

ISSN: 1056-9103

Variables of Interest

Data collected from employee health records included employee age, vaccine type, dates of vaccine administration, date of COVID-19 diagnosis, hospitalization status, date of symptom onset, and antibody test results. The Genomic Pathology Clinical Laboratory collected variables including viral lineage, clade, cycle threshold, and percentage of non-N bases.

Sequencing

Nasopharyngeal swabs that tested positive at Jefferson Occupational Health were transferred to the Molecular and Genomic Pathology Laboratory at TJUH for further evaluation. Viral ribonucleic acid was extracted from the nasopharyngeal specimens using the Biomérieux EasyMag Extraction System, following the standard instrument guidelines (generic protocol). Whole-genome sequencing for SARS-CoV-2 was subsequently performed using the Illumina COVIDSeq protocol (Illumina Inc., San Diego, California), following the manufacturer's guidelines. Remaining specimens were sequenced using the Swift Normalase Amplicon Panels Core Kit along with the SARS-CoV-2 Additional Genome Coverage Primer Panel, following the manufacturer's protocol. For data analysis, Illumina's Local Run Manager's GenerateFASTQ module was used to generate the fastq files for all specimens. The fastq files were uploaded to the DRAGEN bioinformatics analysis pipeline in Illumina Basespace for sequencing quality assessment and COVID-19 lineage identification.

Serologic Testing

Serum antinucleocapsid (anti-N) and antispike (anti-S) antibody data were collected for 74 HCWs at the time of diagnosis. For the measurement of both antibodies, the Roche Elecsys assay was used. Levels less than 0.8 U/mL were considered negative, and levels greater than or equal to 0.8 U/mL were considered positive as per the platform's system specifications. Only qualitative data (positive vs negative) were recorded in the study database.

Statistics

For all employees who experienced breakthrough cases, clinical data were described using simple counts and proportions. These data were stratified by type of vaccine received. The association between type of vaccine and other clinical features was evaluated using Fisher exact tests ($\alpha = 0.05$). Clinical data for all employees who experienced breakthrough cases were merged with sequencing data from the Molecular and Genomic Pathology Clinical Laboratory. For employees for whom sequencing data were available, simple counts, proportions, medians, and interquartile ranges were used to describe subjects, stratified by the strain of the variant with which they were infected. The association of variant strain and clinical characteristics was tested using either the Fisher exact test or the Wilcoxon rank sum test ($\alpha = 0.05$).

RESULTS

Demographics and Clinical Status

During the study period, 520 HCWs were found to have a SARS-CoV-2 breakthrough infection using the nasopharyngeal reverse transcriptase-polymerase chain reaction test. Demographics, clinical status at the time of diagnosis, and need for hospitalization were stratified according to what vaccine the patients received (Table 1).

Among all HCWs with a breakthrough infection, 233 had received 2 doses of the mRNA-1273 vaccine and 287 had received 2 doses of the BNT162b2 vaccine. There was no significant difference noted among the ages of the patients with breakthrough infection depending on the type of vaccine they received ($P = 0.814$). Most patients in both the mRNA-1273 (222 of 233) and in the BNT162b2 group (276 of 287) were symptomatic at the time of diagnosis. However, only 0.85% of the patients with breakthrough infection who were fully vaccinated with mRNA-1273 required hospitalization, whereas none of the patients previously vaccinated with BNT162b2 required admission.

Antibody Testing

A total of 74 plasma samples were collected and tested for anti-N and anti-S antibodies (Table 1). Of the 74 samples collected, all but 2 tested positive for anti-S antibodies (1 sample in each group). Anti-S antibodies were found in 97.8% of the patients who received the BNT162b2 and in 96.6% of those who received the mRNA-1273 vaccine. Anti-N antibodies were noted in 58.6% of HCWs vaccinated with mRNA-1273 and in 57.8% of HCWs vaccinated with BNT162b2 ($P = 0.805$).

COVID Strains and Viral Load

Of the 520 HCWs who experienced breakthrough infection, 164 had sequencing data available. Two individuals had Iota strains and were excluded. Of the remaining 162 individuals, 142 were infected with isolates identified as Delta strains, whereas 20 were infected with isolates identified as Alpha strains (Table 2). There was no association noted between the variant isolated and the type of vaccine the HCWs had received ($P = 0.474$). In symptomatic patients, the median number of days between last vaccination date and initial symptoms was 83 days for patients noted to have the Alpha strain and 202 days for those infected with the Delta strain ($P < 0.001$).

Using both gene N1 and gene N2 primers, cycle thresholds were obtained from the sequenced samples (Table 2). The cycle threshold was significantly lower for HCWs infected with the Delta strain compared with HCWs infected with the Alpha strain ($P = 0.0144$ for gene N1 and $P = 0.0271$ for gene N2). This reflects a significantly higher viral burden at the time of diagnosis for individuals infected with the Delta strain.

DISCUSSION

In this study, we evaluated SARS-CoV-2 breakthrough infections among HCWs in a 950-bed center city hospital in Philadelphia, Pennsylvania, from March 1, 2021, to October 31, 2021. All of the 520 HCWs who tested positive for SARS-CoV-2 were fully vaccinated with 2 doses of an mRNA vaccine—either the Pfizer BNT162b2 or the Moderna mRNA-1273 vaccine. Although mRNA-1273 (100 μg) and BNT162b2 (30 μg) dosing, vaccine composition, and interval between doses are different, no difference in the rate of detectable anti-S antibodies or serious breakthrough infection was found. This supports similar vaccine response and clinical efficacy in HCWs. Furthermore, the breakthrough infections did not generally seem to be severe. Among the entire study population, only 2 patients required hospital admission. This reinforces the previous finding that, despite the acquisition of breakthrough infections, vaccinated individuals experience less severe disease.[2,16,18] Such findings may be used to educate and bolster the confidence of unvaccinated individuals in the advantages of vaccination.

We also observed that, at the time of diagnosis, almost all vaccinated patients had developed anti-S antibodies, whereas slightly more than half had developed anti-N antibodies. From this study, it is difficult to know how protective either antibody is against disease. Because almost all of the patients had developed anti-S antibodies and almost all did well clinically, it is possible that their

TABLE 1. Characteristics of HCWs With Breakthrough COVID-19 Cases by Vaccine

	Moderna (n = 233), Total Antibody Tested (n = 29)	Pfizer (n = 287), Total Antibody Tested (N = 45)	*P* Value for Fisher Exact Test
Age, n (% of population, by vaccine)			
<20 y	0 (0.0)	3 (0.8)	0.814
20–29 y	54 (24.0)	70 (25.0)	
30–39 y	65 (26.3)	86 (30.0)	
40–49 y	47 (20.4)	50 (17.1)	
50–59 y	43 (20.0)	51 (17.9)	
60–69 y	18 (6.6)	21 (7.9)	
>70 y	6 (3.0)	6 (1.7)	
Symptomatic at time of breakthrough diagnosis, n (% of population, by vaccine)	222 (95.2)	276 (96.2)	0.509
If antibody-tested, positive anti-N antibody, n (% of tested, by vaccine)	17 (58.6)	26 (57.8)	0.805
If antibody-tested, positive anti-S antibody, n (% of tested, by vaccine)	28 (96.6)	44 (97.8)	1.000
Hospitalized, n (% of population, by vaccine)	2 (0.85)	0 (0.0)	0.200

development is important for reducing disease severity. Conversely, the development of anti-N antibodies does not seem to be an important factor for protecting against disease. Most patients did well clinically despite their absence in almost half of patients. However, future studies with non-breakthrough controls will be necessary to definitively evaluate these conclusions. Moreover, the role of cellular immunity will need to be addressed in future studies because it has been previously shown to play an important role in infected individuals.[19]

The study has other limitations. We focused on a population in 1 location, which raises concerns regarding geographic generalizability. Furthermore, this study may not be generalizable in regard to underlying health. The individuals are relatively young compared with the general population. Although data regarding the individuals' comorbidities were not collected, they are sufficiently fit to work in a healthcare setting, and thus, their clinical outcomes may not be applicable to the population at large. In addition to control data, as noted previously, future studies will need to collect broader information regarding demographics and medical comorbidities for the improved modeling of outcomes. Furthermore, modeling may be improved with an extended duration of follow-up. Our limited follow-up may have missed patients who might have progressed to severe disease or developed a change in their serological status.

In summation, we can conclude that vaccination remains an important preventive measure to protect patients from severe COVID infection. The Pfizer and Moderna mRNA vaccines seem equally effective in minimizing the severity of breakthrough infections. Serologic patterns suggest that anti-S antibodies may be critical for protection, although further studies will be needed to test for this association. In addition, a significantly higher viral burden at the time of diagnosis was noted for individuals infected with the Delta strain. This may reflect the waning protection of vaccination for emerging strains of the virus.

TABLE 2. Clinical Characteristics, by Variant, of SARS-CoV-2–Infected Individuals Using Merged Occupational Health and Sequencing Data

	Alpha (n = 20)	Delta (n = 142)	*P* Value for Fisher Exact Test or Wilcoxon Rank Sum Test
Symptomatic at breakthrough, n (% by strain)			
No	0 (0.0)	5 (3.5)	1.000
Yes	20 (100.0)	137 (96.5)	
Vaccine received, n (% by strain)			
mRNA-1273 (Moderna)	8 (40.0)	73 (51.4)	0.474
BNT162b2 (Pfizer)	12 (60.0)	69 (48.6)	
Cycle threshold at the time of diagnosis, by gene target, median number of cycles (IQR)			
Gene N1	23.9 (21.2–27.4)	20.8 (18.0–25.4)	0.0144
Gene N2	24.0 (21.5–26.5)	21.0 (18.2–25.7)	0.0271
In patients who were symptomatic, days from last vaccine to date of symptom onset, median (IQR)	83 (71–93)	202 (169–233)	<0.001
In patients who were symptomatic, days from symptom onset to presentation to occupational health, median (IQR)	2 (1–3)	1 (1–3)	0.264

IQR indicates interquartile range.

REFERENCES

1. Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA COVID-19 vaccine in a nationwide mass vaccination setting. *N Engl J Med*. 2021;384(15): 1412–1423.
2. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N Engl J Med*. 2020;383: 2603–2615.
3. Farinholt T, Doddapaneni H, Qin X, et al. Transmission event of SARS-CoV-2 Delta variant reveals multiple vaccine breakthrough infections. *BMC Med*. 2021;19:255.
4. Thompson MG, Burgess JL, Naleway AL, et al. Interim estimates of vaccine effectiveness of BNT162b2 and mRNA-1273 COVID-19 vaccines in preventing SARS-CoV-2 infection among health care personnel, first responders, and other essential and frontline workers—eight US locations, December 2020–March 2021. *MMWR*. 2021;70(13):495–500.
5. Angel Y, Spitzer A, Henig O, et al. Association between vaccination with BNT162b2 and incidence of symptomatic and asymptomatic SARS-CoV-2 infections among health care workers. *JAMA*. 2021;325:2457–2465.
6. Ioannou P, Karakonstantis S, Astrinaki E, et al. Transmission of SARS-CoV-2 variant B.1.1.7 among vaccinated health care workers. *Infect Dis (Lond)*. 2021;53:876–879.
7. Wang R, Chen J, Hozumi Y, et al. Emerging vaccine-breakthrough SARS-CoV-2 variants. *ArXiv*. 2021;8:546–556.
8. Mathema B, Chen L, Chow K, et al. Postvaccination SARS-COV-2 among health care workers in New Jersey: A genomic epidemiological study. *Microbiology Spectrum*. 2021;9(3):e01882–21.
9. World Health Organization. Classification of Omicron (B.1.1.529): SARS-CoV-2 variant of concern. Available at: https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sarscov-2-variant-of-concern. Updated November 26, 2021. Accessed December 23, 2021.
10. Musser JM, Christensen PA, Olsen RJ. Delta variants of SARS-CoV-2 cause significantly increased vaccine breakthrough COVID-19 cases in Houston, Texas. *Am J Pathol*. 2022;192:320–331.
11. Bergwerk M, Gonen T, Lustig Y, et al. COVID-19 breakthrough infections in vaccinated health care workers. *N Engl J Med*. 2021;385:1474–1484.
12. Kustin T, Harel N, Finkel U, et al. Evidence for increased breakthrough rates of SARS-CoV-2 variants of concern in BNT162b2-mRNA–vaccinated individuals. *Nat Med*. 2021;27:1379–1384.
13. Geysels D, Van Damme P, Verstrepen W, et al. SARS-CoV-2 vaccine breakthrough infections among healthcare workers in a large Belgian hospital network. *Infect Control Hosp Epidemiol*. 2021;1–2.
14. Accorsi EK, Britton A, Fleming-Dutra KE, et al. Association between 3 doses of mRNA COVID-19 vaccine and symptomatic infection caused by the SARS-CoV-2 Omicron and Delta variants. *JAMA*. 2022;327(7): 639–651.
15. Sharma P, Mishra S, Basu S, et al. Breakthrough infection with SARS-CoV-2 and its predictors among healthcare workers in a medical college and hospital complex in Delhi, India. *medRxiv*. 2021. doi:10.1101/2021.06.07.21258447.
16. Shamier MC, Tostmann A, Bogers S, et al. Virological characteristics of SARS-CoV-2 vaccine breakthrough infections in health care workers. *medRxiv*. 2021. doi:10.1101/2021.08.20.21262158.
17. Rovida F, Cassaniti I, Paolucci S, et al. SARS-CoV-2 vaccine breakthrough infections with the Alpha variant are asymptomatic or mildly symptomatic among health care workers. *medRxiv*. 2021;12:6032.
18. Butt AA, Nafady-Hego H, Chemaitelly H, et al. Outcomes among patients with breakthrough SARS-CoV-2 infection after vaccination. *Int J Infect Dis*. 2021;110:353–358.
19. Ni L, Ye F, Cheng ML, et al. Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals. *Immunity*. 2020;52(6):971–977.