In-depth Characterization of Vaccine Breakthrough Infections With SARS-CoV-2 Among Health Care Workers in a Dutch Academic Medical Center

Lidewij W. Rümke,1 Femke C. Groenveld,1 Yvonne M. G. van Os,1 Patrice Praest,1 Anniek A. N. Tanja,1 Dorien T. C. M. de Jong,1 Jori Symons,1 Rob Schuurman,1 Tessa Reinders,1 L. Marije Holstra,1 Stefan Nierkens,1 Steven F. T. Thijsen,1 Michiel Heron,1 Robert-Jan Lebbink,1 Jeffrey M. Beekman,5,6 Monique Nijhuis,1 and Annemarie M. J. Wensing1

1Virology, Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, the Netherlands, 2Occupational Health Office, Department of Human Resources, University Medical Center Utrecht, Utrecht, the Netherlands, 3Center for Translational Immunology, University Medical Center Utrecht, Utrecht, the Netherlands, 4Department of Medical Microbiology & Immunology, Utrecht, the Netherlands, 5Department of Pediatric Pulmonology, Wilhelmina Children’s Hospital, University Medical Center, Utrecht, the Netherlands, and 6Regenerative Medicine Center Utrecht, University Medical Center, Utrecht University, Utrecht, the Netherlands

Severe acute respiratory syndrome coronavirus 2 infection after coronavirus disease 2019 vaccination raises concerns about the emergence of vaccine escape variants. Here we characterize 14 breakthrough infections among 5860 fully vaccinated Dutch health care workers ≥14 days after the final dose of vaccination with either BNT162b2, mRNA-1273, or Ad26.COV2.S. These breakthrough infections presented with regular B.1.1.7 (Alpha) and B.1.617.2 (Delta) variants and high viral loads, despite normal vaccine-induced B- and T-cell immune responses detected by live virus neutralization assays and ELISPOT. High-risk exposure settings, such as in households, indicate a potential risk of viral transmission despite full vaccination.

Keywords. SARS-CoV-2; COVID-19; vaccine breakthrough; postvaccination infection; immunity.

To counter the coronavirus disease 2019 (COVID-19) pandemic, multiple severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein-based vaccines have been developed. Two mRNA (BNT162b2, Pfizer-BioNTech; mRNA-1273, Moderna) and 2 adenoviral vector vaccines (ChAdOx1 nCoV-19, Astra-Zeneca; Ad26.COV2.S, Johnson & Johnson/Janssen) are currently authorized for use in the European Union [1]. These vaccines have proved safe and highly effective in preventing severe COVID-19 [2]. The emergence of novel SARS-CoV-2 variants with mutations in the spike gene raised concerns about increased transmissibility and escape of vaccine-induced immunity. Indeed, breakthrough infections in fully vaccinated individuals have been reported [3–5]. Limited information is available on the immunological protection against the specific variants in these cases and settings in which breakthrough infections occur. Here we describe a clinical, epidemiological, and virological characterization of 14 fully vaccinated health care workers (HCWs) with subsequent SARS-CoV-2 breakthrough infection, with additional longitudinal in-depth immunological characterization in 3 subjects.

METHODS

Sample Collection and Study Population
COVID-19 vaccination of University Medical Center Utrecht (UMCU) staff started in January 2021. The SARS-CoV-2 testing policy included a polymerase chain reaction (PCR) test in symptomatic HCWs (since April 2020) and in asymptomatic HCWs after unprotected exposure to SARS-CoV-2-infected individuals (since December 2020). Vaccine breakthrough infection was defined as a positive SARS-CoV-2 PCR ≥14 days after receiving Ad26.COV2.S or the second dose of BNT162b2 or mRNA-1273. Information on demographics, comorbidities, vaccination, disease severity, and exposure to SARS-CoV-2-infected individuals was collected.

Patient Consent Information
Subject characteristics and combined naso-/oropharyngeal swabs were collected as part of the standard protocol for SARS-CoV-2 testing of UMCU staff. All subjects approved use of their data for the purpose of scientific research. Immunological characterization was performed in 3 subjects in comparison to SARS-CoV-2-naïve controls who all participated in an observational study approved by the UMCU Institutional Review Board (ABR NL73903.041.20).

Real-time Quantitative PCR and Viral Sequencing
Real-time quantitative PCR (RT-qPCR) was performed on combined naso-/oropharyngeal swabs with the Allplex 2019-nCoV Assay (Seegene, Seoul, South Korea), targeting the E (Envelope), N (Nucleocapsid), and RdRp (RNA-dependent RNA polymerase) genes. A positive result was defined as amplification up to 45 cycles of any of the SARS-CoV-2 genes.

RNA was extracted on the Hamilton MicroLab StarLET using the STARMag 96 X 4 Universal Cartridge Kit (Seegene, Seoul, South Korea). Whole-genome sequencing of SARS-CoV-2 was
performed on the Ion Torrent Genexus Integrated Sequencer (ThermoFisher Scientific, Waltham, Massachusetts) with the Ion Torrent GX5 Chip and the Ion AmpliSeq SARS-CoV-2 Research Panel according to an edited system-installed assay, “Ion AmpliSeq SARS-CoV-2 Research Assay,” with a minimum read count per sample of 500,000. Ion Torrent Genexus Software (version 6.2.1, ThermoFisher Scientific) using the IRMAReport plugin for genome-assisted assembly of the consensus sequence and the COVID19AnnotateSnpEff plugin (version 1.3.0.2) was used for sequence analysis and variant annotation. The IRMA consensus sequences were uploaded in the Pangolin Web Application (version 3.0.5, lineages version 2021-06-05) for lineage assignment. Nextclade (version 1.1.0) was used for phylogenetic analysis of the IRMA consensus sequences under the terms and conditions of the Creative Commons (CC BY) license (https://creativecommons.org/licenses/by/4.0/). The sequences have been submitted to GenBank (accession numbers: OK356625-OK356635, OL548845, OL555796, and OL672887).

Antinucleocapsid and Antispike Immunoglobulin G
Peripheral blood was obtained from 3 HCWs at diagnosis (resp. days 6, 1, and 0 after symptom onset) and in the convalescent phase (resp. days 27, 21, and 20 after symptom onset). Two commercial immunoassays were applied. The Abbott SARS-CoV-2 Alinity-i is a semiquantitative chemiluminescent microparticle immunoassay (CMIA) targeting antinucleocapsid immunoglobulin G (IgG; Abbott Laboratories, Abbott Park, Illinois, USA). An index value of ≥1.4 is considered positive. The DiaSorin Liaison SARS-CoV-2 TrimericS IgG chemiluminescence immunoassay (CLLA) quantifies IgG antibodies against a trimeric S-protein antigen on a DiaSorin Liaison (DiaSorin, Stillwater, Minnesota, USA), expressed as binding antibody units (BAU/mL). Samples with values of ≥33.8 BAU/mL are considered positive.

SARS-CoV-2 Neutralization Assay
Live virus neutralization assays were performed as previously described [6] using the SARS-CoV-2 strain/NL/2020 (EVAg-010V-03903) and a clinical B.1.1.7 (Alpha) strain. Serum samples were heat-inactivated, serially-diluted, and mixed with 120TCID50 SARS-CoV-2. These serum-virus mixtures were incubated for 1 hour (37°C) and then applied to Vero E6 cells. After 2.5 days of incubation (37°C), supernatant was collected for SARS-CoV-2 E gene RT-qPCR. The 50% inhibitory dilutions (ID50) were calculated by linear interpolation using the mean of duplicate responses. All laboratory procedures using live SARS-CoV-2 were performed in a biosafety level 3 facility.

SARS-CoV-2 ELISpot Assay
To measure SARS-CoV-2-specific T-cell reactivity, an in-house developed ELISpot was performed, similar to a previously described procedure except for the addition of SARS-CoV-2 peptide pools [7]. Peripheral blood mononuclear cells (PBMCs) were isolated using a Ficoll density gradient. Per sample, 6 wells of an ELISpotPRO plate precoated with polyvinylidene difluoride (Mabtech, Nacka Strand, Sweden) were used to stimulate 100 µL of 2.5 × 106 PBMCs/mL with 50 µL of mitogen control (antihuman CD3 monoclonal antibody CD3-2 [0.1 µg/mL]; Mabtech, Nacka Strand, Sweden), a negative control (AIM-V medium, Invitrogen, Carlsbad, California, USA), and 4 PepTivator SARS-CoV-2 lyophilized peptide pools (15-mer sequences with 11 amino acids overlap: Prot_S [immunodominant sequence domains of the S glycoprotein], Prot_S1 [N-terminal S1 domain of the S glycoprotein], Prot_M [complete sequence of membrane glycoprotein {M}], and Prot_N [complete sequence of nucleocapsid phosphoprotein {N}]; Miltenyi Biotec, Bergisch Gladbach, Germany; GenBank MN908947.3; protein QHD43416.1, QHD43419.1, QHD43423.2, QHD43416.1]). SARS-CoV-2-specific IFN-y-secreting T cells/2.5 × 105 PBMCs were measured using an ELISpot Reader (Autoimmun Diagnostika GmbH, Straβberg, Germany).

RESULTS
From January to May 2021, 1396 HCWs were vaccinated with 2 doses of BNT162b2 (January and February 2021), 1714 HCWs with 2 doses of mRNA-1273 vaccine (April and May 2021), and 2740 HCWs with a single dose of Ad26.COV2.S (April and May 2021). Among these HCWs, 14 breakthrough infections (0.2%) were reported between March and June 2021 (estimated follow-up of 12 800 person-months). Cases presented 18–111 days (median, 57 days) after final vaccination (Supplementary Figure 1). The median age (range) was 47 (26–62) years, and 13 (93%) were women (Table 1); this is in line with the overall population of UMCU staff. Three subjects reported underlying disease (resp. asthma, atopic dermatitis, and Hashimoto’s thyroiditis). Three were asymptomatic, and 11 had mild to moderate disease. None required hospitalization. Cycle threshold (Ct) values of SARS-CoV-2 RT-qPCR on combined naso-/oropharyngeal swabs ranged from 17.8 to 31.9 for the E gene (median, 23.6), 19.6 to 35.1 for the N gene (median, 25.9), and 19.0 to 35.4 for the RdRp gene (median, 25.1). Ten subjects self-reported a household member as a possible index case. All subjects were isolated at home early in infection; no secondary cases could be ascertained.

Examination of SARS-CoV-2 sequences revealed B.1.1.7 (Alpha) in 12 and B.1.617.2 (Delta) in 2 subjects. All contained the amino acid changes in the spike gene characteristic for these variants [8]; only the B.1.1.7 (Alpha) sequence of subject 13 lacked the N501Y mutation. Eight contained additional spike mutations (Table 1). Phylogenetic analyses indicated that these
Table 1. Clinical Characteristics, Vaccine History, and Sequencing Results of 14 Health Care Workers With SARS-CoV-2 Vaccine Breakthrough Infection

| Case | Sex | Age, y | Contact With Patients | Underlying Disease | Immuno-compromised | Vaccine Type | Days From Completed Vaccination to Symptom Onset | Days From Completed Vaccination to Positive PCR | Symptoms | Possible Index Case (Self-Reported) | Ct Value (E, N, RdRp gene) | Sequence-Based Typing | Additional Mutations Spike Gene |
|------|-----|--------|-----------------------|--------------------|---------------------|--------------|-----------------------------------------------|-----------------------------------------------|----------|---------------------------------|-------------------------|---------------------------|-----------------------------|
| 1    | Female | 45 | Yes | No | No | BNT162b2 | 42 | 43 | Anosmia, arthralgia, fever, headache, myalgia, peripheral neuropathy, rhinosinusitis | Partner | 18.5, 19.9, 20.6 | Alpha (B.1.17) | - |
| 2    | Female | 62 | Yes | No | No | BNT162b2 | 78 | 78 | Anosmia, rhinosinusitis | Partner | 23.7, 27.3, 24.9 | Alpha (B.1.17) | - |
| 3    | Female | 27 | Yes | No | No | BNT162b2 | 63 | 64 | Rhinitis | Unknown | 23.5, 25.9, 25.4 | Alpha (B.1.17) | A771V |
| 4    | Female | 52 | Yes | Asthma | No | BNT162b2 | 60 | 61 | Cough, dyspnea, fever | Unknown | 178, 19.6, 19.0 | Alpha (B.1.17) | - |
| 5    | Female | 35 | Yes | No | No | BNT162b2 | 72 | 74 | Anosmia, cough, rhinitis | Partner | 21.5, 24.0, 22.6 | Alpha (B.1.17) | H245Y |
| 6    | Female | 35 | Yes | No | No | BNT162b2 | 77 | 80 | Anosmia, rhinosinusitis | Partner, son | 24.7, 26.9, 26.7 | Alpha (B.1.17) | S494P |
| 7    | Male | 58 | Yes | No | No | BNT162b2 | N/A | 80 | Asymptomatic | Partner | 31.9, 35.1, 33.8 | Alpha (B.1.17) | - |
| 8    | Female | 26 | Yes | No | No | BNT162b2 | 110 | 111 | Fever, rhinitis | Friend | 19.8, 21.8, 21.3 | Alpha (B.1.17) | V382L |
| 9    | Female | 38 | Yes | No | No | Ad26. COV2.S | 32 | 38 | Cough, fever, pharyngitis, rhinosinusitis | Colleague | 18.9, 20.2, 20.2 | Alpha (B.1.17) | D68V |
| 10   | Female | 57 | Yes | No | No | Ad26. COV2.S | 35 | 37 | Cough, dyspnea | Daughter | 21.5, 25.0, 22.7 | Alpha (B.1.17) | V483I, A706V |
| 11   | Female | 50 | Yes | No | No | Ad26. COV2.S | N/A | 20 | Asymptomatic | Son | 23.6, 25.9, 25.9 | Alpha (B.1.17) | S12F, D906N |
| 12   | Female | 54 | No | Atopic dermatitis | No | Ad26. COV2.S | 47 | 45 | Cough, fever, headache, myalgia, otitis | Partner | 31.3, 34.5, 35.4 | Delta (B.617.2) | - |
| 13   | Female | 38 | Yes | Hashimoto’s thyroiditis | No | Ad26. COV2.S | N/A | 18 | Asymptomatic | Partner | 29.0, 31.5, 31.1 | Alpha (B.1.17) | - |
| 14   | Female | 48 | Yes | No | No | Ad26. COV2.S | 50 | 52 | Anosmia, fever, headache, myalgia, rhinosinusitis | Daughter | 29.2, 313, 32.4 | Delta (B.617.2) | G142D |

Abbreviations: Ct, cycle threshold; E, Envelope; N, Nucleocapsid; PCR, polymerase chain reaction; RdRp, RNA-dependent RNA polymerase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.  
*Defined as the day of the second dose of BNT162b2 or the day of the single dose of Ad26.COVID2.  
Characteristic amino acid mutations in the spike encoding gene Alpha variant (B.1.17: delB370, del145, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H), and Delta variant (B.617.2: T19R, del157/158, L452R, T478K, D614G, P681R, D90N).  
*No N501Y mutation detected.
**Figure 1.** Phylogenetic analysis, live virus neutralization, and ELISpot T-cell assays of health care workers with SARS-CoV-2 breakthrough infection. A. A maximum likelihood phylogenetic tree of 14 SARS-CoV-2 vaccine breakthrough sequences (depicted in bold). Viral sequences obtained from subjects 12 and 14 clustered with the Delta branch (green); the other sequences clustered with the Alpha branch (blue). In the background are 1900 publicly available sequences, representing the ongoing global pandemic (GISAID). B. Live virus neutralization assay against the original Dutch strain (NL/2020) and an Alpha (B.1.1.7) strain in sera of a fully vaccinated, SARS-CoV-2-naive
Peripheral blood was available from subjects 1, 2, and 12. All antinucleocapsid IgG assays were negative at diagnosis (resp. 0.01, 0.02, and 0.03) and positive in the convalescent phase (resp. 2.75, 4.01, and 5.40). The antispike IgG assays were positive both at diagnosis (resp. 1830, 667, and 80.6 BAU/mL) and in the convalescent phase (resp. >2080, >2080, and >2080 BAU/mL).

At diagnosis, neutralizing antibody titers against the original Dutch (NL/2020) and B.1.1.7 (Alpha) strains were in the same range as vaccinated controls (Figure 1B). A 10-fold increase in neutralizing activity against the original Dutch strain was seen in the convalescent phase in all 3 subjects and against the B.1.1.7 (Alpha) strain in subjects 1 and 2 (Figure 1B). Subjects 1 and 2 showed strong T-cell reactivity early after symptom onset (resp. days 6 and 1) against the spike peptide pools (resp. 131 and 51 spot-forming cells [SFC]), comparable to BNT162b2 vaccinated controls. In contrast to these controls, subjects 1 and 2 also showed reactivity against the membrane and nucleocapsid peptide pools (resp. 9 and 45 SFC) (Supplementary Figure 2).

In subject 12, vaccinated with Ad26.COV2.S, only 3 SFC were detected on the day of symptom onset. During the convalescent phase, this HCW showed a strong increase in T-cell reactivity against all peptide pools (77 SFC) (Figure 1C).

**DISCUSSION**

We describe 14 fully vaccinated, healthy individuals with breakthrough infections 18–111 days after full COVID-19 vaccination. Independent of the presence of symptoms, several had low Ct values, reflecting a high viral load and potential infectiousness [9].

Breakthrough infections are expected to occur due to ineffective vaccine-elicted immune responses, waning immunity, or escape of immune recognition by viral evolution. The SARS-CoV-2 sequences of our cases resembled the circulating VOCs in the general Dutch population at the time of sample collection, with B.1.1.7 (Alpha) accounting for 84% of the total number of Dutch infections in March 2021 and the emergence of B.1.617.2 (Delta) in June 2021 [10]. In 1 subject infected with B.1.1.7 (Alpha), sequencing revealed an additional spike mutation, S494P, associated with higher binding affinity toward the ACE2 receptor and was predicted as a possible vaccine escape mutation [11, 12].

Although our study is limited by the lack of available blood samples of all participants, in-depth immunological characterization of 3 cases did not point to absence of vaccine-induced antibody responses. Within the first week after symptom onset, all had high levels of antispike IgG and antibodies with virus-neutralizing capacity. Three weeks later, seroconversion of antinucleocapsid IgG and an increase of neutralizing antibody titers were seen. As spike IgG is generally elicited from day 7 after symptom onset [13], this pattern is very suggestive of a serological spike-based vaccine response combined with a broader serological response by natural infection in the convalescent phase. In subjects 1 and 2, high T-cell reactivity was present early after symptom onset, which decreased to similar levels as vaccinated controls after 3 weeks. This might be explained by a booster effect of natural infection on the T-cell response elicited earlier by vaccination, followed by a retraction phase in which T cells are regulated. In subject 12, vaccinated with 1 dose of Ad26COV2.S, T-cell reactivity at the day of symptom onset was low and increased after infection. All subjects showed systemic vaccine responses. It can be questioned to what extent current vaccines also induce local immune responses [14]. Unfortunately, we have no information regarding the immune responses in the respiratory tracts of the subjects.

Our observations show that even potent, systemic vaccine-induced immune responses can be insufficient, particularly in high-exposure scenarios. Ten of 14 HCWs self-reported exposure to a SARS-CoV-2-positive household member. These interactions typically coincide with prolonged and high-density contact, which includes the early stage of infection of the index, in which viral load and transmissibility risk are highest [15]. Although current Dutch public health policies advise avoiding contact with infected persons, vaccinated individuals might rely on vaccine-induced immunity and be less cautious within the household setting. Our observations highlight that in high-risk exposure settings caution is warranted and physical distancing from SARS-CoV-2-infected individuals should be maintained regardless of vaccination status.

It should be stressed that although COVID-19 vaccination has proven highly effective, breakthrough infections will occur in a proportion of vaccinated individuals [3]. The exact percentage of breakthrough infections in this cohort should be interpreted with caution. As our HCWs were not tested regularly after vaccination regardless of symptoms, asymptomatic cases may have been missed. Also, no breakthrough cases after
vaccination with mRNA-1273 were identified, but the follow-up period after vaccination with this vaccine was only 1 month, compared with 5 for BNT162b2 and 1–2 months for Ad26. COV2.S vaccinees. Therefore, we refrained from presenting epidemiological data per vaccine and will continue to monitor this cohort, especially in light of the current emergence of B.1.617.2 (Delta). Overall, this report underlines the importance of large-scale vaccine programs and should in no way undermine public confidence in mass vaccination.

In conclusion, the frequency of SARS-CoV-2 breakthrough infection after full vaccination is currently low and could not be directly attributed to viral escape or lack of vaccine-induced immune response in our cases. Several of our 14 cases presented with high viral loads and were potentially infectious to others. The majority of infections occurred after self-reported exposure to a SARS-CoV-2-positive household member. Our observations support the need to test symptomatic or exposed vaccinated HCWs and raise awareness to maintain physical distancing for vaccinated individuals from persons with COVID-19-like symptoms, particularly in high-density contact scenarios such as household settings.

Acknowledgments

We would like to thank all study participants and Lauke Boeijen, Shannon la Grouw, Robert van de Kieft, Rosa Elias, and Max Hulsman for their assistance in the project.

Financial support. This work was supported by Health–Holland (grant number LSHM20058) and ZonMw (grant number 114025009).

Potential conflicts of interest. L.M.H. received a presentation honorarium from Janssen Pharmaceutica. M.N. received consulting fees from the Gilead Advisory Board. L.M.H. and A.M.J.W. received kits for a SARS-CoV-2 antigen validation study from Abbott. A.M.J.W. received kits for HIV drug level determination from ARK Diagnostics. A.M.J.W. received consulting fees for advisory activities on antiviral drugs from Gilead, ViIV, and GSK and support for attending meetings and a fee for accredited educational lectures from Virology Educations. All payments were made to the University Medical Center Utrecht. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Author contributions. L.R. and F.G. collected data, performed data analysis, and wrote the manuscript; Y.v.O. and T.R. collected data and performed data analysis; P.P., A.T., D.T.C.M.J., J.S., S.F.T., and M.H. performed experiments and data analysis; R.S., L.M.H., S.N., R.B.J., and J.M.B. aided in interpreting the results and provided critical feedback; M.N. and A.W. were involved in the design and supervision of this study and the writing of the manuscript. All authors approved the final manuscript version to be submitted.

References

1. COVID-19 vaccines. European Medicines Agency. Available at: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/covid-19-vaccines Accessed 22 July 2021.
2. Pormohammad AZM, Ghorbani S, Mohammadi M, et al. Efficacy and safety of COVID-19 vaccines: a systematic review and meta-analysis of randomized clinical trials. Vaccines 2021; 9:467.
3. CDC COVID-19 Vaccine Breakthrough Case Investigations Team. COVID-19 vaccine breakthrough infections reported to CDC – United States, January 1–April 30, 2021. MMWR Morb Mortal Wkly Rep 2021; 70:792–3.
4. Hacisuleyman E, Hale C, Saito Y, et al. Vaccine breakthrough infections with SARS-CoV-2 variants. N Engl J Med 2021; 384:2212–8.
5. McEwen AE, Cohen S, Bryson-Cahn C, et al. Variants of concern are overrepresented among post-vaccination breakthrough infections of SARS-CoV-2 in Washington State [manuscript published online ahead of print 24 June 2021]. Clin Infect Dis 2021.
6. Algaissi A, Hashem AM. Evaluation of MERS-CoV neutralizing antibodies in sera using live virus microneutralization assay. Methods Mol Biol 2020; 2099:107–16.
7. Thijssen S, Heron M, Greemels H, et al. Elevated nucleoprotein-induced interferon-γ release in COVID-19 patients detected in a SARS-CoV-2 enzyme-linked immunosorbent spot assay. J Infect 2020; 81:452–82.
8. Alaa Abdel Latif JLM, Alkuwzemy M, Tsuang G, et al. SARS-CoV-2 (hCoV-19) mutation reports. B.1.17 and B.1.617.2 lineage reports. Available at: https://outbreak.info/situation-reports. Accessed 13 July 2021.
9. Singanayagam A, Patel M, Charlett A, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Euro Surveill 2020; 25:2001483.
10. Rijksinstituut voor Volksgezondheid en Milieu (RIVM). Varianten van het coronavirus SARS-CoV-2. Available at: https://www.rivm.nl/coronavirus-covid-19/virus/varianten. Accessed 22 July 2021.
11. Wang R, Chen J, Gao K, Wei GW. Vaccine-escape and fast-growing mutations in the United Kingdom, the United States, Singapore, Spain, India, and other COVID-19-devastated countries. Genomics 2021; 113:2158–70.
12. Chakraborty S. Evolutionary and structural analysis elucidates mutations on SARS-CoV2 spike protein with altered human ACE2 binding affinity. Biochem Biophys Res Commun 2021; 534:374–80.
13. Liu X, Wang I, Xu X, et al. Patterns of IgG and IgM antibody response in COVID-19 patients. Emerg Microbes Infect 2020; 9:1269–74.
14. Schieffelin JS, Norton EB, Kolls JK. What should define a SARS-CoV-2 “breakthrough” infection? J Clin Invest 2021; 131:e151186.
15. Cheng HY, Jian SW, Liu DP, et al; Taiwan COVID-19 Outbreak Investigation Team. Contact tracing assessment of COVID-19 transmission dynamics in Taiwan and risk at different exposure periods before and after symptom onset. JAMA Intern Med 2020; 180:1156–63.