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BRIEF REPORT

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

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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-naive 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

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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 (CLIA) 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, serial-diluted, and mixed with 120 TCID50 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 (PMBCs) were isolated using a Ficoll density gradient. Per sample, 6 wells of an ELISpot^{PRO} plate precoated with polyvinylidene difluoride (Mabtech, Nacka Strand, Sweden) were used to stimulate 100 µL of 2.5 × 10^6 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 lyophylized 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-γ-secreting T cells/2.5 × 10^5 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	Immunocompromised	Vaccine Type	Days From Completed Vaccination[a] to Symptom Onset	Days From Completed Vaccination[a] to Positive PCR	Symptoms	Possible Index Case (Self-Reported)	Ct Value (E, N, RdRp gene)	Sequence-Based Typing[b]	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.1.1.7)	-
2	Female	62	Yes	No	No	BNT162b2	78	78	Anosmia, rhinosinusitis	Partner	23.7, 27.3, 24.9	Alpha (B.1.1.7)	-
3	Female	27	Yes	No	No	BNT162b2	63	64	Rhinitis	Unknown	23.5, 25.9, 25.4	Alpha (B.1.1.7)	A771V
4	Female	52	Yes	Asthma	No	BNT162b2	60	61	Cough, dyspnea, fever	Unknown	17.8, 19.6, 19.0	Alpha (B.1.1.7)	-
5	Female	35	Yes	No	No	BNT162b2	72	74	Anosmia, cough, rhinitis	Partner	21.5, 24.0, 22.6	Alpha (B.1.1.7)	H245Y
6	Female	35	Yes	No	No	BNT162b2	77	80	Anosmia, rhinosinusitis	Partner, son	24.7, 26.9, 26.7	Alpha (B.1.1.7)	S494P
7	Male	58	Yes	No	No	BNT162b2	N/A	80	Asymptomatic	Partner	31.9, 35.1, 33.8	Alpha (B.1.1.7)	-
8	Female	26	Yes	No	No	BNT162b2	110	111	Fever, rhinitis	Friend	19.8, 21.8, 21.3	Alpha (B.1.1.7)	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.1.1.7)	D88V
10	Female	57	Yes	No	No	Ad26.COV2.S	35	37	Cough, dyspnea	Daughter	21.5, 25.0, 22.7	Alpha (B.1.1.7)	V483I, A706V
11	Female	50	Yes	No	No	Ad26.COV2.S	N/A	20	Asymptomatic	Son	23.6, 25.9, 25.9	Alpha (B.1.1.7)	S12F, D905N
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.1.1.7)[c]	-
14	Female	48	Yes	No	No	Ad26.COV2.S	50	52	Anosmia, fever, headache, myalgia, sinusitis	Daughter	29.2, 31.3, 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.

[a]Defined as the day of the second dose of BNT162b2 or the day of the single dose of Ad26.COV2.S.

[b]Characteristic amino acid mutations in the spike encoding gene Alpha variant (B.1.17: del69/70, del145, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H), and Delta variant (B.617.2: T19R, del157/158, L452R, T478K, D614G, P681R, D950N).

[c]No N501Y mutation detected.

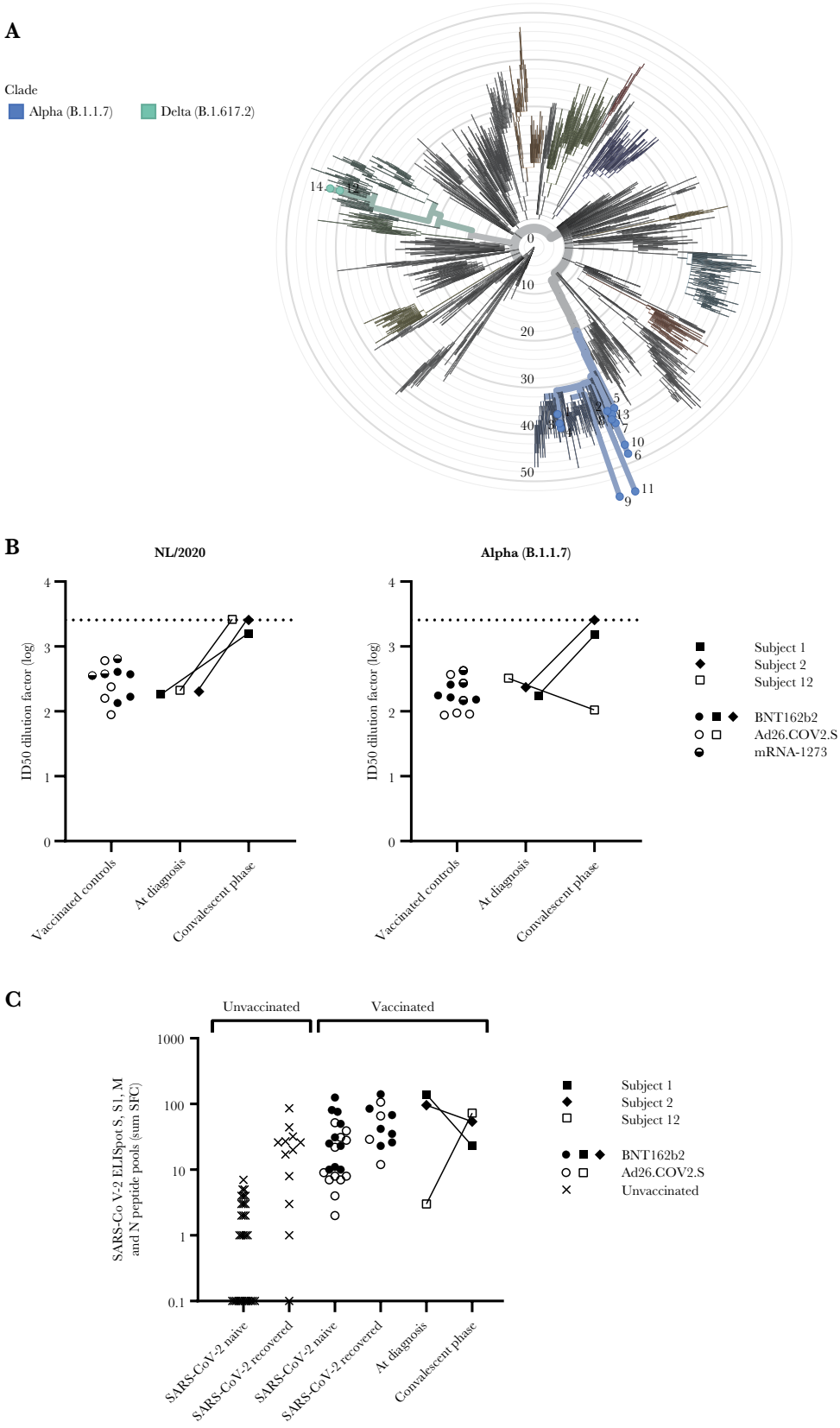

Figure 1. Phylogenetic analysis, live virus neutralization, and ELISpot T-cell assays of health care workers with SARS-CoV-2 breakthrough infection. A, A maximum likelihood phylogenetic tree of 14 SARS-CoV-2 vaccine breakthrough sequences (depicted in bold). Viral sequences obtained from subjects 12 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

sequences clustered with circulating sequences in the Dutch population and did not form a separate cluster (Figure 1A).

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-elicited 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

control group (BNT162b2 [closed circles], mRNA-1273 [semicircles], or Ad26.COV2.S [open circles] vaccine) and 3 health care workers with SARS-CoV-2 breakthrough infection after full vaccination with BNT162b2 (full squares) and Ad26.COV2.S (open squares). The sera of subjects 1, 2, and 12 were collected 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). The dotted line represents the upper detection limit. C, SARS-CoV-2-specific T-cell reactivity against S, S1, M, and N peptide pools measured by the sum of SFC in a unvaccinated SARS-CoV-2-naive control group, an unvaccinated SARS-CoV-2-recovered control group, a fully vaccinated SARS-CoV-2-naive control group (BNT162b2 [closed circles] or Ad26.COV2.S [open circles] vaccine), a fully vaccinated control group with previous SARS-CoV-2 infection, and 3 health care workers (subjects 1, 2 and 12) with SARS-CoV-2 vaccine breakthrough infection, sampled 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). Abbreviations: M, membrane; N, nucleocapsid; S, spike; S1, spike1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFC, spot-forming cells.

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.

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