Outbreak of SARS-CoV-2 B.1.1.7 Lineage after Vaccination in Long-Term Care Facility, Germany, February–March 2021


Outbreaks of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in long-term care facilities (LTCF) are of great concern and have been reported to have high case-fatality rates (1). Consequently, national vaccination strategies prioritize residents of LTCFs (2).

The coronavirus disease (COVID-19) mRNA vaccine BNT162b2 (Pfizer-BioNTech, https://www.pfizer.com) has demonstrated high efficacy against COVID-19 (3). Protection has been observed ≥12 days after the first vaccination, and reported vaccine efficacy is 52% between the first and second dose and 91% in the first week after the second dose (3). Although breakthrough infections have been reported, vaccinated persons were at substantially lower risk for infection and symptomatic disease (4,5).

The variant of concern (VOC) B.1.1.7 rapidly became the predominant lineage in Europe in 2021. Analyses estimated that B.1.1.7 has increased transmissibility and a ≤0.7 higher reproduction number (6). Neutralization activity of serum samples from BNT162b2-vaccinated persons has been shown to be slightly reduced against B.1.1.7 in cell culture (7), but observational data from Israel suggest BNT162b2 vaccination is effective against B.1.1.7 (8).

We investigated a SARS-CoV-2 B.1.1.7 outbreak in a LTCF, which involved 20 BNT162b2-vaccinated residents and 4 unvaccinated residents. We report on clinical outcomes, viral kinetics, and control measures applied for outbreak containment. The study was approved by the ethics committee of Charité–Universitätsmedizin Berlin (EA2/066/20) and conducted in accordance with the Declaration of Helsinki and guidelines of Good Clinical Practice (https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e6-r2-guideline-good-clinical-practice-step-5_en.pdf).

The Study

On February 4, 2021, daily SARS-CoV-2 screening of employees yielded a positive antigen point-of-care test (AgPOCT) result in 1 caregiver in a LTCF in Berlin, Germany. Among 24 residents of the unit under their responsibility, 20 (83%) residents had received the second dose of BNT162b2 on January...
29 or 30, 2021 (Figure 1). Four residents had not been vaccinated for nonmedical reasons (i.e., personal refusal or delayed provision of consent by legal guardian). AgPOCTs and reverse transcription PCR (RT-PCR) testing of all residents on February 4 detected SARS-CoV-2 infections in 3/4 unvaccinated and 10/20 vaccinated residents (Figure 1). At the time of testing, 2 vaccinated patients exhibited mild fatigue and one of those also had diarrhea; all other patients were asymptomatic.

The next week, testing detected 7 additional infections, resulting in 4/4 unvaccinated infected residents and 16/20 vaccinated infected residents. The remaining 4 vaccinated residents tested negative throughout the 30-day observation period (Figure 1).

In addition to residents, 11/33 (33%) staff members from the unit tested positive for SARS-CoV-2 by February 18; of those, none were twice-vaccinated staff members, 2/8 (25%) had received 1 dose of BNT162b, and 9/22 (40.9%) had not been vaccinated. No infected staff required hospital treatment.

Respiratory symptoms, including cough and shortness of breath, occurred in 5/16 (31.3%) vaccinated patients and all 4 unvaccinated patients (Figure 2, panel A; Appendix Table, https://wwwnc.cdc.gov/EID/article/27/8/21-0887-App1.pdf). All 4 unvaccinated SARS-CoV-2–infected patients and 2/16 (12.5%) vaccinated patients required hospitalization (Figure 1; Figure 2, panel A). Supplemental oxygen therapy was required by 3/4 (75.0%) unvaccinated and 1/16 (6.3%) vaccinated patients (Figure 1; Figure 2, panel A). Two patients, 1/16 (6.3%) vaccinated persons and 1/4 (25.0%) unvaccinated persons, required intermittent oxygen therapy after discharge. One vaccinated patient with a history of hypertension and microvascular dementia died 6 days after testing positive by RT-PCR because of a hypertensive crisis with intracerebral hemorrhage. Another vaccinated patient died 16 days after testing positive by RT-PCR. Neither patient experienced respiratory symptoms during the infection (Figure 1).

Containment measures in place included mandatory use of FFP2 or N95 masks and daily AgPOCT screening for anyone entering the facility. Immediately after detection, the facility was closed to visitors and additional containment measures were put in place, including designated staff and separate entrance, elevator, and changing rooms. Staff were required to change personal protective equipment before entering each room. Residents of all 7 units of the LTCF underwent weekly AgPOCT for ≥3 weeks, and residents in the adjacent unit underwent AgPOCT every 2–3 days. The outbreak was contained within the unit; no further cases were detected.

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**Figure 1.** Individual trajectories of 24 long-term care facility residents over 30-day study period in outbreak of SARS-CoV-2 B.1.1.7 lineage infections, Germany, February–March 2021. A) Four unvaccinated residents; B) 20 residents who received their second dose of BNT162b2 COVID-19 mRNA vaccine (https://www.pfizer.com) on January 29 or 30, 2021. After a positive result in a healthcare worker, residents received AgPOCT and subsequently underwent regular RT-PCR testing for SARS-CoV-2. Dotted lines indicate respiratory symptoms, and continuous lines indicate hospitalization. AgPOCT, antigen point-of-care test; COVID-19, coronavirus disease; RT-PCR, reverse transcription PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
Outbreak of SARS-CoV-2 B.1.1.7 after Vaccination

All SARS-CoV-2 RNA-positive samples were tested for presence of SARS-CoV-2 VOCs by RT-PCR and complete genome sequencing (Appendix). RT-PCR suggested the presence of B.1.1.7, which was confirmed by sequencing in 11 patients for whom sufficient sequence information was available. In phylogenetic analysis, sequences form a monophyletic clade with additional sequences from Berlin interspersed (Appendix Figure 1), suggesting a common outbreak source, including infections outside the unit.

We performed serial RT-PCR testing of nasopharyngeal swab specimens from 22 patients. SARS-CoV-2 RNA concentrations peaked within 5 days (Appendix Figure 2). The median peak SARS-CoV-2 RNA concentration in vaccinated and unvaccinated patients overlapped concentrations detected at time of diagnosis in B.1.1.7 patients of similar
ages (Figure 2, panel B). However, SARS-CoV-2 RNA concentration was lower among vaccinated residents than unvaccinated residents, although the difference was not statistically significant (6.45 vs. 8.15 log_{10} copies/mL; p = 0.10). Furthermore, duration of SARS-CoV-2 RNA shedding was considerably shorter in vaccinated patients than in unvaccinated patients (7.5 [95% CI 7–17.3] days vs. 31 [95% CI 21.5–34.5] days; p = 0.003) (Figure 2, panel C). Peak SARS-CoV-2 RNA concentrations above 10^{6} copies per mL, below which virus isolation in cell culture is usually not successful, were detected in all 4 unvaccinated patients but only in 7/16 vaccinated patients (9).

We further assessed the level of infectiousness in 22 samples from 14 patients by virus cell culture (Appendix). One sample obtained from a vaccinated patient 7 days after the first positive RT-PCR test, which showed 9.32 log_{10} SARS-CoV-2 RNA copies/mL, yielded a positive isolation outcome. Isolation attempts from samples of the same patient taken in the next 4 days and from 21 samples taken from 13 other patients were unsuccessful.

Five weeks after initial testing, 8/8 vaccinated and infected residents and 2/2 unvaccinated and infected residents showed robust antibody responses against SARS-CoV-2 spike antigens, virus neutralization capacity, and interferon-γ release of SARS-CoV-2–specific T cells (Figure 2, panels D, E; Appendix Figure 3). These results confirm the immune response capability in these patients.

Conclusions
We performed a longitudinal study of SARS-CoV-2 infections in a LTCF unit. Nearly all infected residents were symptomatic, including most residents that had received a second BNT162b2 dose the week before. The outbreak was caused by SARS-CoV-2 VOC lineage B.1.1.7, which might partly explain the high attack rate and lack of protection in vaccinated residents. Nevertheless, we reported a lower attack rate, a shorter duration of SARS-CoV-2 RNA shedding, and a lower proportion of symptomatic COVID-19 requiring hospitalization and oxygen support for vaccinated patients. However, despite the limited sample size and the short interval between second vaccination and infection, this outbreak raises questions about the effectiveness of the vaccination regimen in the elderly (3,8,10–12). A delayed and overall reduced immune response to BNT162b2 vaccination has been described in elderly persons (13,14), which might explain the reported outbreak and infections in LTCF described elsewhere (4,5).

This outbreak highlights that older adults have reduced protection ≤2 weeks after second BNT162b2 vaccination. Therefore, single-dose regimens and extended dosing intervals might be insufficient for fully protecting this population (15). Vaccination of LTCF residents and staff is likely effective in reducing the spread of SARS-CoV-2. However, regular SARS-CoV-2 screening, prompt outbreak containment, and nonpharmaceutical interventions (16) remain necessary for optimal protection in this setting.

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V.M.C. is named together with Euroimmun GmbH on a patent application filed recently regarding SARS-CoV-2 diagnostics through antibody testing.

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References


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Appendix

Patients, Materials and Methods

Subjects were enrolled in the Pa-COVID-19 study conducted at Charité - Universitätsmedizin Berlin, a prospective observational study on the pathophysiology of coronavirus disease (COVID-19) (1). The Pa-COVID-19 study is registered in the German and the World Health Organization international registry for clinical studies (DRKS00021688). Written informed consent was obtained from all patients or legal representatives. Additional investigation of nasopharyngeal and oropharyngeal swabs was performed in accordance with §25 of the Berlin State Hospital Law, allowing for pseudonymized analysis of routine patient data by the treating physicians.

SARS-CoV-2 Screening

We performed rapid antigen point-of-care tests (AgPOCT) (NADAL COVID-19 Ag Schnelltest [Nal von minden, https://www.nal-vonminden.com] and MEDsan SARS-CoV-2 Antigen Rapid Test [MaiMed, https://maimed.de]) (2), and patients affected by the outbreak received regular oropharyngeal or nasopharyngeal swabs (daily, every 2–3 days, or weekly, depending on the patients’ willingness) throughout the study period. Swab specimens were immediately stored in 2 mL of viral transport medium at −20°C.

Real-Time Reverse Transcription PCR for SARS-CoV-2 and Typing PCR

RNA was extracted by using the MagNApur 96 DNA and viral NA small volume Kit (Roche, https://www.roche.com) on a MagNA Pure 96 System as recommended by the manufacturer. Real time reverse transcription PCR (rRT-PCR) was performed targeting the envelope (E) gene and nucleocapsid (N) gene on the Roche Light Cycler 480 system (Tib-Molbiol, https://www.tib-molbiol.de). Viral loads in throat swab specimens were given as
SARS-CoV-2 copies/mL diluted swab specimen. rRT-PCR used targets in the E and N genes on the Roche Light Cycler 480 system (Tib-Molbiol). Viral loads in throat swab specimens were presented as SARS-CoV-2 copies/mL diluted swab. Assessment of SARS-CoV-2 RNA concentration was done by applying external or internal calibration curves and quantified SARS-CoV-2 RNA and by using serial diluted specific in vitro–transcribed RNA standards as previously described (3–5). A probe-based melting curve assay (Tib-Molbiol) for SARS-CoV-2 was used to screen for single nucleotide polymorphisms in the spike gene (leading to amino acid changes N501Y and del69/70) associated with variants of concern such as B.1.1.7; both changes were detected (6).

**Antibody Assessment and IFN-γ Release of SARS-CoV-2–Specific T Cells**

For detection of SARS-CoV-2–specific antibodies to the spike and nucleocapsid proteins, we used a microarray-based multiparameter immunoassay according to manufacturer’s instructions (SeraSpot Anti-SARS-CoV-2 IgG, Seramun Diagnostica GmbH, https://www.seramun.com), as described elsewhere (7). We applied a commercially available IGRA for assessment of IFN-γ release of SARS-CoV-2–specific T cells according to manufacturer’s instructions (Euroimmun, https://www.euroimmun.com), as described previously (7).

**Virus Isolation**

Virus isolation was performed by using Vero E6 cells (ATCC CRL-1586). Vero E6 cells were maintained in a 5% CO₂ atmosphere at 37°C and cultured in Dulbecco’s Modified Eagle Medium (Sigma Aldrich, https://www.sigmaaldrich.com), supplemented with 10% fetal bovine serum, 1% non-essential amino acids 100x concentrate, and 1% sodium pyruvate 100 mM (ThermoFisher Scientific, https://www.thermofisher.com) and split twice a week. Vero E6 cells were seeded at a density of 175,000 cells per well in 24-well plates 1 day before isolation. Virus isolation experiments were performed under Biosafety Level 3 (BSL-3) conditions with enhanced respiratory personal protective equipment. For virus isolation, the medium was removed and cells were rinsed once with 1x phosphate buffered saline (ThermoFisher Scientific) and inoculated with 200 µL of swap sample. After 1 hour incubation at 37°C, 800 µL of isolation medium (supplemented with 2% FBS, 1% penicillin-streptomycin, and 1% amphotericin B) was added to each well. Cells were monitored for cytopathic effect (CPE) for the following 3 days. As soon as CPE was visible or at day 3 post inoculation, viral RNA was quantified from the
supernatant of the inoculated cells. To ensure that viruses with lower replication capacities were not missed, all cultures were cultivated for an additional 3 days. At 6 days after inoculation, all cultures were reexamined for CPE. No CPE was visible in any negatively tested culture. In addition, all supernatants were passaged once by inoculating fresh and confluent Vero E6 cells with 100 µL of cell culture supernatant (taken at 3 days postinoculation) from the respective samples and monitored as previously described. For isolation of viral RNA, 50 µL of supernatant was diluted in 300 µL of MagNA Pure 96 external lysis buffer (Roche). All samples were heat inactivated for 10 minutes at 70°C before export from the BSL-3. Isolation and purification of viral RNA was performed using the MagNA Pure 96 System (Roche) according to the manufacturer recommendations. Viral RNA was quantified by using rRT-PCR (E gene assay) as previously described in Corman et al. (4). Positive isolation success was determined when CPE was visible and viral RNA concentrations were above a threshold of 100,000 genome equivalents per µL.

**High-Throughput Sequencing of SARS-CoV-2 Genomes**

Sufficient sample material (SARS-CoV-2 RNA concentration >10^4 copies/mL) was available for sequencing for 14 patients. We applied a PCR amplicon-based sequencing approach by using random hexamers and the SuperScript III Reverse transcription kit (ThermoFisher Scientific) according to manufacturer’s instructions after a PCR amplification using the primer sets (V3) published by the Artic Network (https://github.com/artic-network/artic-ncov2019) (8). We set up a 25 µL PCR master mix by using the Q5 High-Fidelity DNA Polymerase kit (New England Biolabs, https://www.neb.com) with 5 µL 5x Q5 Reaction Buffer, 13.15 µL RNase-free water, 0.5 µL 10 mM dNTPs, 3.6 µL of either 10 µM primer pool 1 or 2, 2.5 µL cDNA and 0.25 µL Q5 High-Fidelity DNA Polymerase. We performed PCR by using a thermocycling protocol with initial denaturation at 98°C for 30 sec, followed by 35 cycles of 98°C for 15 sec, 65°C for 5 min, followed by a final 2-min extension step at 72°C. PCR products were pooled and purified by using KAPA Pure Beads (Roche) according to manufacturer’s instructions. We used up to 5 ng DNA of purified PCR amplicons and the KAPA Frag Kit, followed by HTS library preparation using the KAPA Hyper Prep Kit (Roche) according to manufacturer’s instructions. Sequencing was done using the V3 chemistry (2x75bp) on the Illumina NextSeq platform (Illumina, https://www.illumina.com).
Statistics

Values are given as medians and interquartile range unless stated otherwise. GraphPad PRISM statistics version 27.0 (IBM Deutschland, https://www.ibm.com/de-de) was used for statistical analysis. Group differences were assessed in a univariate analysis by using Fisher exact test or nonparametric Mann Whitney U test. P values of <0.05 were considered statistically significant. All 95% CI for proportions were calculated by using the Wilson procedure with correction for continuity (9).

Bioinformatics

Reads were trimmed by using AdapterRemoval version 2.3.0 and aligned to the Wuhan-Hu-1 (GenBank Accession no. MN908947.2) reference sequence using bowtie2 (version 2.4.1). Consensus calling used ivar version 1.9 requiring a coverage of ≥3 reads per position, and a minimum frequency threshold of 0.6. Lineages were assigned using pangolin version 2.3.5 (https://github.com/hCoV-2019/pangolin). A phylogenetic tree was inferred from an alignment generated in MAFFT version 7.471, including all complete sequences from the outbreak as well as representative B.1.1.7 sequences from Berlin deposited in GISAID, using IQTree version 2.0.3 with a GTR substitution model and 10000 ultra-fast bootstrap replicates. Sequences are available on GISAID under accession numbers EPI_ISL_1635432 (H07), EPI_ISL_1635435 (H09), EPI_ISL_1635666 (H11), EPI_ISL_1635964 (H12), EPI_ISL_1636216 (H14), EPI_ISL_1636365 (H15), EPI_ISL_1636400 (H17), EPI_ISL_1636401 (H18), EPI_ISL_1636403 (H22), and EPI_ISL_2134632 (H25).

References

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**Appendix Table.** Patient characteristics in outbreak of severe acute respiratory syndrome coronavirus 2 B.1.1.7 lineage after vaccination in a long-term care facility, Germany, February–March 2021*  

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients</th>
<th>Vaccinated patients</th>
<th>Unvaccinated patients</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>24 (100.00)</td>
<td>20 (83.33)</td>
<td>4 (16.67)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>19 (79.17)</td>
<td>15 (75.00)</td>
<td>4 (100.00)</td>
<td>0.544</td>
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<tr>
<td>M</td>
<td>5 (20.83)</td>
<td>5 (25.00)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>90 (75–105)</td>
<td>90.5 (75–105)</td>
<td>88.5 (84–94)</td>
<td>0.735</td>
</tr>
<tr>
<td>Underlying conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>17 (70.83)</td>
<td>15 (75.00)</td>
<td>2 (50.00)</td>
<td>0.552</td>
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<tr>
<td>Type 2 diabetes</td>
<td>7 (28.17)</td>
<td>6 (30.00)</td>
<td>1 (25.00)</td>
<td>1.000</td>
</tr>
<tr>
<td>COPD</td>
<td>5 (20.83)</td>
<td>4 (20.00)</td>
<td>1 (25.00)</td>
<td>1.000</td>
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<tr>
<td>Chronic kidney disease</td>
<td>10 (41.67)</td>
<td>7 (35.00)</td>
<td>3 (75.00)</td>
<td>0.272</td>
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<tr>
<td>Dementia</td>
<td>12 (50.00)</td>
<td>10 (50.00)</td>
<td>2 (50.00)</td>
<td>1.000</td>
</tr>
<tr>
<td>Other</td>
<td>20 (83.33)</td>
<td>16 (80.00)</td>
<td>4 (100.00)</td>
<td>1.000</td>
</tr>
<tr>
<td>Infected</td>
<td>20 (83.33)</td>
<td>16 (80.00)</td>
<td>4 (100.00)</td>
<td>1.000</td>
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<tr>
<td>Outcome in infected patients</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Respiratory symptoms</td>
<td>9 (45.00)</td>
<td>5 (31.25)</td>
<td>4 (100.00)</td>
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<tr>
<td>Hospitalization</td>
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<td>2 (12.5)</td>
<td>4 (100.00)</td>
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<td>Oxygen in hospital</td>
<td>4 (20.00)</td>
<td>1 (6.25)</td>
<td>3 (75.00)</td>
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<td>Oxygen after discharge</td>
<td>2 (10.00)</td>
<td>1 (6.25)</td>
<td>1 (25.00)</td>
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<td>Death</td>
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<td>2 (12.5)</td>
<td>0</td>
<td>1.000</td>
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<td>Virologic examinations, median (IQR)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak virus concentration, RNA (copies/mL, log_{10})</td>
<td>6.62 (5.55–7.92)</td>
<td>6.45 (4.58–7.17)</td>
<td>8.15 (6.78–8.81)</td>
<td>0.100</td>
</tr>
<tr>
<td>Time to negative PCR or AgPOCT, d</td>
<td>10 (7–24)</td>
<td>7.5 (7–17.25)</td>
<td>31 (21.50–34.50)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Values are no. (%) except as indicated. AgPOCT, antigen point-of-care test; COPD, chronic obstructive pulmonary disease; IQR, interquartile range.
Appendix Figure 1. Maximum likelihood phylogenetic tree showing the sequences associated with outbreak of severe acute respiratory syndrome coronavirus 2 B.1.1.7 lineage in long-term care facility in relation to other B.1.1.7 sequences circulating in Berlin, January–February 2021. Complete genome sequences from the outbreak (n = 10) are in blue; additional sequences from Berlin are in black. For 4 samples, sequencing failed. Labels are given to sequences not associated with the outbreak if they fall into the same clade as the outbreak sequences. The x-axis shows substitutions per site. Asterisks indicate nodes with bootstrap support >70 and nodes with bootstrap support ≤30 are shown as polytomies. The tree is rooted with an early sequence from Wuhan (GISAID accession no. EPI_ISL_402125). The complete sequences from 6 patients are identical (H07, H11, H12, H17, H22, and H25). One patient had a mutation not present in any other sequence (H15) and 4 patients had a variable genome sequence position with a nucleotide that was present either as a major (H09, H14, H18) or a minor (H17) variant. The new mutation in H15 is likely the result of sporadic mutation (10); the variant present (sometimes as a minority) in 4 patients may be indicative of direct transmission links within the unit.
Appendix Figure 2. SARS-CoV-2 RNA viral concentration/mL of diluted swab specimens from vaccinated (n = 20) and unvaccinated (n = 4) residents of long-term care facility over 30 days, Germany, February–March 2021. Assessment of SARS-CoV-2 RNA concentration was done by applying external or internal calibration curves and quantified SARS-CoV-2 RNA and by using serial diluted specific in-vitro transcribed RNA. Unvaccinated residents are shown in blue. LLQ, lower limit of quantification; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
Appendix Figure 3. Anti-SARS-CoV-2 S1, full spike, and nucleocapsid IgG antibody response and surrogate virus neutralization test after vaccination in study of outbreak of SARS-CoV-2 B.1.1.7 lineage after vaccination in long-term care facility, Germany, February–March 2021. A) Anti-SARS-CoV-2 S1, B) full spike, and C) nucleocapsid-specific IgG antibodies were measured in 10/20 (50.00%) vaccinated and 2/4 (50.00%) unvaccinated residents 5 weeks after initial testing. D) Neutralizing capacity of antibodies was measured using the ELISA-based surrogate virus neutralization test cPASS (medac GmbH, https://international.medac.de). Unvaccinated patients are shown in blue. ACE2, angiotensin-converting enzyme 2; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S/CO, signal-to-cutoff ratio.