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Article

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Abstract

Since little is known about viral and host characteristics of breakthrough infections after COVID-19 vaccination, a nationwide investigation of breakthrough cases was initiated in Japan. 130 cases (90%+ received mRNA vaccines) were reported with respiratory specimens in 117 cases and sera in 68 cases. A subset of cases shed infectious virus regardless of symptom presence or viral lineages. Viral lineages for breakthrough infections matched both temporally and spatially with the circulating lineages in Japan with no novel mutations in spike receptor binding domain that may have escaped from vaccine-induced immunity were found. Anti-spike/neutralizing antibodies of breakthrough infections in the acute phase owing to vaccine-induced immunity were significantly higher than those from unvaccinated convalescent individuals but were comparable to vaccinated uninfected individuals, and followed by boosting in the convalescent phase. Symptomatic cases had low anti-spike/neutralizing antibodies in the acute phase with robust boosting in the convalescent phase, suggesting the presence of serological correlate for symptom development in COVID-19 vaccine breakthrough infections.

Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in significant morbidity and mortality globally¹, but multiple effective vaccines have been developed and authorized for use at an unprecedented speed²⁻⁴. In Japan, BNT162b2 (Pfizer/BioNTech) was initially approved on February 14, 2021 with two doses three weeks apart, and roll-out started for healthcare workers on February 17, 2021⁵. mRNA-1273 (Moderna) was approved on May 21, 2021 with two doses four weeks apart, and roll-out has started for the elderly. AZD1222 (AstraZeneca) was also approved but is not yet administered under the Immunization Act. As of June 30, 2021, almost all healthcare workers, 37.1% of people over 65, and 9.3% of the general population had been vaccinated twice⁶. Since several approved vaccines have extraordinarily high efficacy²⁻⁴, there has been scarce opportunity to study viral and host characteristics in breakthrough cases. Therefore, since late March 2021, the National Institute of Infectious Diseases (NIID) in Japan has initiated a nationwide COVID-19 vaccine breakthrough investigation with 3 objectives: (1) to study viral and host characteristics in breakthrough cases, (2) to detect mutations and/or variants that may have escaped from vaccine-induced immunity or emerged under selective pressure, and (3) to detect outbreaks among vaccinees. Here we present a report of 130 cases identified in this investigation. A standardized case report form (CRF) was used to collect information on patients' characteristics. Respiratory samples were collected for viral isolation, PCR screening for N501Y, E484K, and L452R mutations, and viral genome sequencing. Sera were also collected to detect antibody response against vaccination and subsequent infection.

Results

Case characteristics

136 cases were reported during the study period; 4 cases were excluded because specimens for diagnosis were collected <14 days after the first dose, and 2 cases were excluded for having no vaccination date for the first dose. Thus, the final analysis included 130 cases (63 partially vaccinated and 67 fully vaccinated) from 27 of the 47 prefectures in Japan. Case characteristics are described in **Table 1**. Median age was 44.5 (range 20-98) with 37 males (28.5%). No cases had a history of primary/acquired immunodeficiency, but 3 individuals were using immunosuppressants. 121 cases were vaccinated with BNT162b2, 2 with mRNA-1273, 1 with AZD1222 (vaccinated overseas), and 6 with unknown (5 were healthcare workers who likely received BNT162b2). Of the 94 cases vaccinated twice with BNT162b2, 86 (91.5%) had a dose interval between 19-23 days. At the time of CRF submission, 65 (50%) were asymptomatic, 60 (46.2%) were mild, and 5 (3.8%) were moderate/severe (3 requiring oxygen supplementation). None required intubation or treatment in the intensive care unit.

Specimen collection and RT-qPCR

Respiratory specimens were collected for 117 cases (59 fully vaccinated), including 74 nasopharyngeal swabs, 40 saliva samples, and 3 nasal swabs. 94 (80.3%) were samples taken for diagnosis, while others were retaken for this investigation. 75 of the 117 cases (34 fully vaccinated) had detectable SARS-CoV-2 RNA during in-house retest. Quantification cycle (C_q) values were similar between partially vaccinated cases and fully vaccinated cases (**Fig. 1a**). Although the results may be confounded by case demographics, there was no significant difference in viral RNA levels between these two groups in this study.

Viral isolation and viral titer measurement

Among 72 cases (32 fully-vaccinated) with enough samples for viral isolation and titration, 20 (6 fully-vaccinated) had viable virus evaluated by cytopathic effect followed by RT-qPCR confirmation.

Viral titers in the respiratory specimens with viable virus were as high as 3.2×10^4 TCID₅₀/mL with correlation between viral RNA levels and viral titers observed for both fully- and partially-vaccinated cases (**Fig. 1b, and 1c**). There was no apparent difference in viral titers between fully- and partially-vaccinated cases. Of note, 6 individuals who shed infectious virus were asymptomatic and lineages included B.1.1.7 (Alpha variant), B.1.617.2 (Delta variant), and R.1 (includes E484K mutation; neither VOC or variant of interest; WHO nomenclature not designated).

PCR screening for mutations of interest and viral RNA genome sequencing

RT-qPCR screening for N501Y, E484K, or L452R mutations was done for 75 cases (**Table 2**). Viral genome sequencing was successfully performed for 48 cases (19 fully vaccinated): 34 cases of B.1.1.7 (Alpha variant), 9 cases of B.1.617.2 (Delta variant), 4 cases of R.1, and 1 case of P.1 (Gamma variant) were found (**Table 2**). Confirmed lineages based on sequencing and presumed lineages inferred from mutation-specific PCR were plotted by week of specimen collection of 68 cases for all of Japan (**Extended Data Fig. 1**), as well as for Eastern Japan (**Fig. 1d**) and Western Japan (**Fig. 1f**). We compared these breakthrough lineages with lineages detected in samples submitted to the COVID-19 Genomic Surveillance Network in Japan (COG-JP), which is a consortium of public health institute, airport quarantine stations, and NIID. COG-JP has a wide geographical coverage in Japan, but cases of B.1.1.7 (Alpha variant) are likely to be overrepresented as samples that test positive with RT-qPCR detecting N501Y at public health institutes were sequenced preferentially during the study period. On the other hand, since RT-qPCR detecting L452R screening was expanded in mid-late June, 2021, the proportion of B.1.617.2 (Delta variant) is likely to be underestimated in COG-JP. To complement this, based on L452R screening done at commercial laboratories, the proportion of L452R mutations was estimated to be 37% in Eastern Japan but only 5.8% in Western Japan as of June 30⁷. In Eastern Japan, several lineages were detected among breakthrough cases depending on dominant lineages in each time period (R.1 in late March to early May, B.1.617.2 (Delta variant) after late May, and B.1.1.7 (Alpha variant) for the entire period) (**Fig. 1e**). In Western Japan, as B.1.1.7 (Alpha variant) was introduced in early January and quickly became

dominant (**Fig. 1g**), we only detected B.1.1.7 (Alpha variant) among breakthrough cases. Since there is reasonable concern that the virus may have escaped from vaccine-induced immunity or emerged under selective pressure, we looked at point mutations/deletions in the RBD of the spike protein, which is considered to play a key role in determining antigenicity of the virus⁸. There was no novel mutation in RBD except for lineage-defining mutations (i.e., mutations generally observed in each lineage).

Antibody response in acute and convalescent phase of breakthrough infection

To explore the hypothesis that vaccine breakthrough cases may have insufficient antibody response from vaccination and subsequent infection, we examined antibody response in 68 cases. 21 cases had samples from the acute phase (0-5 days since diagnosis; more accurately, days since collection of the first positive specimen), while 61 cases had samples from the convalescent phase (7-53 days since diagnosis), including 14 cases with paired samples. When antibody response was plotted by time since diagnosis or symptom onset, anti-nucleocapsid (N) antibodies, induced not by mRNA/viral vector-based COVID-19 vaccine but by infection, were not detectable in the acute phase, but gradually increased over time (**Fig. 2a, and 2d**). On the other hand, anti-spike (S) and neutralizing antibodies, which can be induced by both vaccination and infection, were highly detectable from the acute phase of breakthrough infection (**Fig. 2b, 2c, 2e, and 2f**). We further compared antibody response among breakthrough infections with two control groups: unvaccinated individuals who have recovered from asymptomatic or mild infection (19-118 days after first PCR positive results) and vaccinated health workers who have never tested positive with PCR and tested negative for anti-N antibodies (24-38 days after second dose). Anti-N antibodies increased to the same level as unvaccinated convalescent individuals on average (**Fig. 3a**), but small proportion of cases (8 of 34 (26.5%)) did not seroconvert even after 14 days since diagnosis. Anti-S and neutralizing antibodies in the acute phase of breakthrough infection among fully-vaccinated individuals were significantly higher than those from unvaccinated convalescent individuals, but were comparable to post-vaccinated individuals without infection (**Fig. 3b, and 3c**). In the convalescent phase, fully-vaccinated

breakthrough cases had increased titer to a level higher than post-vaccinated individuals without infection. These results held true for breakthrough cases among partially-vaccinated individuals except that the titer in the convalescent phase was higher than post-vaccinated individuals for neutralizing antibodies but not anti-S antibodies (**Extended data Fig. 2**). There was one partially vaccinated individual who did not develop anti-S and neutralizing antibodies in the acute phase but had detectable antibodies in the convalescent phase. Finally, we compared antibody response between asymptomatic and symptomatic breakthrough cases. In the acute phase, anti-S and neutralizing antibodies were lower among symptomatic cases than asymptomatic cases although the difference was not statistically significant (**Fig. 3e, and 3f**). Both anti-S and neutralizing antibodies showed significantly boosting from acute to convalescent phase for symptomatic cases, but not for asymptomatic cases because the titers were already quite high in the acute phase. This was in contrast to anti-N antibody response where significant increase was observed from acute to convalescent phase for both asymptomatic and symptomatic cases (**Fig. 3d**).

Discussion

Here we present a comprehensive assessment of baseline demographics, viral characteristics, and host antibody responses among COVID-19 vaccine breakthrough infections. Fully vaccinated individuals were mostly younger and partially vaccinated individuals were bimodal (younger and elderly), reflecting the priority allocation of vaccines to healthcare workers, followed by the elderly. The male-to-female ratio of breakthrough cases was approximately 1:3, which was comparable to individuals under 65 reported to have been vaccinated between April 12 and April 25, 2021 (presumably healthcare workers)⁶. There were no immunocompromised individuals and only a few cases of immunosuppressant use.

Some respiratory specimens were shown to contain infectious virus even among fully vaccinated individuals and asymptomatic individuals, indicating that the vaccine breakthrough cases have the potential to cause secondary transmission via respiratory droplets regardless of presence/absence of symptoms or viral lineage.

We next found that the virus detected among breakthrough cases matched the viruses circulating both temporally and spatially. Several reports have suggested increased breakthrough rates of SARS-CoV-2 variants of concern and variants with mutations known to escape antibodies *in vitro*⁹⁻¹⁰. In our investigation, when there was major transmission of the R.1 lineage, which contains the E484K mutation (known to escape antibodies *in vitro*¹¹), we saw several breakthrough cases with this lineage. However, once B.1.1.7 (Alpha variant), followed by B.1.617.2 (Delta variant), neither of which contain the E484K mutation, became dominant over time due to their high transmissibility¹², we ceased to see breakthrough cases with R.1 lineages. Instead, we started to observe B.1.1.7 (Alpha variant) and B.1.617.2 (Delta variant), despite the fact that major changes in antigenicity are not expected in these lineages, especially for B.1.1.7 (Alpha variant). Therefore, the occurrence of vaccine breakthrough cases may be defined by the interplay between transmissibility and antigenicity of the variants. The relative importance of antigenicity is likely to become more apparent when a greater proportion of the population is vaccinated.

We also examined individual spike mutations for cases where full viral genome was available as there

has also been a reasonable concern that vaccine may positively select for the virus that escape immunity. Our investigation scheme was robust in detecting novel spike RBD mutations as most breakthrough cases appeared independently during community transmission throughout Japan, but no novel RBD mutation was found besides lineage-defining ones. A few case reports describe novel spike mutations that are not found in respective lineages among vaccinated individuals^{10, 13}, but the likelihood of occurrence of novel RBD mutation may not be high even among breakthrough cases. Finally, we explored the host immune response in breakthrough cases and found that anti-S and neutralizing antibody levels were high from the acute phase of breakthrough infection, which were far more than what was observed for asymptomatic/mild cases in unvaccinated individuals and was comparable with the response seen in vaccinated individuals without infection. This response cannot be explained by infection itself, and indeed, we saw significantly higher antibody levels in the convalescent phase than in vaccinated individuals without infection. Although we were not able to obtain pre-infection serum samples to confirm that these individuals had antibodies before infection, and thus cannot exclude the possibility that these individuals had swift antibody response before diagnosis, these results demonstrate that breakthrough cases can occur among individuals with robust antibody response at least through breakthrough infection and that breakthrough cases were not occurring among vaccine non-responders. We also noted that the anti-N antibodies were not detected in some convalescent sera after breakthrough infection. This may be due to early viral clearance leading to non-seroconversion¹⁴ and suggests that the sole use of anti-N antibodies to detect breakthrough cases may not be suitable. We further observed that symptomatic cases had marginally lower anti-S and neutralizing antibody titers in the acute phase compared to asymptomatic cases with significant increase in titers from acute to convalescent phase to the level comparable to asymptomatic cases. These results collectively suggest that symptomatic cases have insufficient anti-S and neutralizing antibody titer to prevent symptom development in the pre-infection phase. The potential usefulness of neutralizing antibody as a correlate of protection has been implicated^{15, 16}, but further studies exploring antibody response among vaccinated individuals pre- and post-breakthrough infection will be urgently needed.

There are at least five limitations in this study. First, although the study was a public health investigation at the national level, the case series comprised only a part of breakthrough cases in Japan (approximately one-third of fully vaccinated cases notified in HER-SYS). Also, the cases mostly consisted of healthcare workers, and therefore are not representative of the population in Japan. Secondly, as samples were not collected specifically for this investigation, storage and transportation may not have been ideal and some specimens were collected at a later time, resulting in 42 cases (36%) with no detectable viral RNA upon retest at NIID, although low viral RNA load may also be a hallmark of breakthrough cases¹⁷. Thirdly, there was no data on when these individuals were exposed to the virus and the immune status at the time of contact was unknown. Fourthly, we did not assess SARS-CoV-2-specific T-cell response, which contributes to protection when antibody titers are low in non-human primate models¹⁷. Finally, our investigation does not evaluate the effectiveness of COVID-19 vaccines and should not be used to undermine the importance of vaccination, which is a critical tool in the effort to end the COVID-19 pandemic.

Overall, the current study aimed to provide a comprehensive understanding of both viral and host factors. Our results indicate that COVID-19 vaccine breakthrough cases occur through infection of circulating strains even among cases with robust antibody response early in the course of infection. Although we have not detected any novel variant that escaped mutation thus far, we must stay vigilant for emergence of these variants. In order to achieve this, it is important to continue to test suspect cases of SARS-CoV-2 infection regardless of their vaccination status, and to attempt to perform sequencing on a subset of these cases. At the same time, until community transmission subsides, appropriate infection prevention measures, based on recommendations from public health authorities, are warranted for each individual regardless of vaccination status, as the probability of emergence of novel variants of concern increases as the virus has more opportunities to transmit and mutate.

Methods

Ethics statement

Demographic data and respiratory samples were collected as part of public health activity under the Infectious Diseases Control Law. Blood specimens were collected for clinical testing provided by NIID with patient consent and retrospective analysis was done as a research activity with ethics approval (NIID ethics committee approval number 1275). Serology data for recovered cases and post-vaccinated individuals without SARS-CoV-2 infection are also included from other studies (NIID ethics committee approval numbers 1245, 1273). Preliminary results of the public health activity portion (case characteristics/respiratory specimen analysis) were published in Japanese on the MHLW/NIID websites to meet statutory requirements¹⁸.

Case definition, CRF, and specimen collection

In this investigation, a “partially vaccinated” individual was defined as an individual who had a positive result from a test detecting SARS-CoV-2 RNA or antigen on a respiratory specimen collected ≥ 14 days after the first dose when multiple doses are required as a primary series under approved conditions (e.g., BNT162b2 and mRNA-1273). A “fully vaccinated” individual was defined as an individual who had a positive result from a test detecting SARS-CoV-2 RNA or antigen on a respiratory specimen collected ≥ 14 days after the second dose.

On March 30, 2021, an Office Memorandum was released from the Ministry of Health, Labour and Welfare (MHLW) to request healthcare facilities, local health centers, and public health institutes to send specimens from partially vaccinated and fully vaccinated cases to NIID. The Health Center Real-time information-sharing System on COVID-19 (HER-SYS), which is a platform for the legal notification of COVID-19 in Japan, was utilized as support to detect fully vaccinated cases for inclusion in this investigation. Blood specimens could also be submitted concurrently for antibody testing as part of clinical practice upon physicians’ request. An example of the CRF can be found in the **Supplementary Table**. Respiratory specimens can be either sputum, tracheal aspirate, bronchoalveolar lavage fluid, oropharyngeal swab, nasal aspirate, nasal swab, nasopharyngeal swab,

or saliva. Blood specimens were either serum or plasma. Quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) was done at NIID for all respiratory samples received using NIID-N2 primer/probe set¹⁹ to confirm that the sample quality was suitable for subsequent testing. In this report, all cases reported from April 1 to June 30, 2021 were included.

Viral isolation and viral titer measurement

Viral isolation and PCR screening for mutations of interest, specifically N501Y, E484K, and L452R, were attempted for NIID-N2 primer/probe set positive samples. Viral isolation was attempted for all quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) positive cases with available residual specimens as described previously²⁰. Briefly, VeroE6/TMPRSS2 cells were seeded in 96-well flat-bottom plates, respiratory specimen mixed with Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2% FBS and Antibiotic-Antimycotic (Thermo Fisher Scientific, MA USA) was inoculated in duplicate, culture supernatant was changed to fresh medium on 1 day post-infection (d.p.i.) and incubated at 37 °C supplied with 5% CO₂. On 1, 3, and 5 d.p.i., cytopathic effect (CPE) was observed. After 5 d.p.i., supernatant was collected and RT-qPCR using SARS-CoV-2 direct detection RT-qPCR kit (Takara Bio, Shiga, Japan) was performed to confirm the propagation of SARS-CoV-2. For samples with indeterminate RT-qPCR results, blind passage was done for further attempt at isolation. Median tissue culture infectious dose (TCID₅₀) in the residual specimens was determined for all viral isolation positive cases.

SARS-CoV-2 viral RNA genome sequencing

For respiratory specimens with adequate RNA, whole genome sequencing of SARS-CoV-2 was attempted. Whole genome sequences of SARS-CoV-2 were obtained by PrimalSeq protocol to enrich cDNA of the SARS-CoV-2 genome by multiplex RT-qPCR amplicons using a modified version of the multiplexed PCR primer set which was originally proposed by Wellcome Trust ARTIC Network²¹⁻²². Briefly, PCR products were purified and subjected to Illumina library construction using QIAseq FX DNA Library Kit (QIAGEN, Hilden, Germany). NextSeq 500 platform (Illumina, San Diego, CA,

USA) was used for sequencing the indexed libraries. The NGS reads were mapped to the SARS-CoV-2 Wuhan-Hu-1 reference genome sequence (29.9 kb ss-RNA; GenBank ID: MN908947), resulting in the specimen-specific SARS-CoV-2 genome sequence by fully mapping on the reference. These mapped reads of SARS-CoV-2 sequences were assembled using A5-miseq v.20140604 to determine the full genome sequence. The SNV sites and marked heterogeneity were extracted by read-mapping at $\geq 10 \times$ depth and the region from 99 to 29,796 nt of the Wuhan-Hu-1 genome sequence.

Serology

We used three assays to detect vaccine-induced and infection-induced antibodies. First, we used widely used commercial electrochemiluminescence immunoassays to detect binding antibodies against nucleocapsid protein (Elecsys Anti-SARS-CoV-2 (Roche)) and receptor binding domain of spike protein (Elecsys Anti-SARS-CoV-2 S (Roche)). A cutoff value of 0.8 U/mL as determined by the manufacturer was used to determine the presence/absence of anti-spike antibodies. A cutoff value determined by the manufacturer is 1.0U/mL, but a different cutoff of 0.128 as proposed by Public Health England was used for anti-nucleocapsid antibodies (both cutoffs are shown in the figures)²³. With these cutoffs, assays have adequate sensitivity (N: 99.4% against PCR-positive samples 20+ days post-symptom onset and S: 97.7% against PCR-positive samples 14+ days post-symptom onset) and specificity (N: 98.1% and S:100% both against PCR-negative samples) through external evaluation by Public Health England²⁴⁻²⁵. Past reports also provide insight into the usefulness of detecting anti-spike antibodies among individuals vaccinated with BNT162b2 using Elecsys Anti-SARS-CoV-2 S (Roche)²⁶.

We also conducted authentic virus neutralization assay to detect functional antibodies. Briefly, a mixture of 100 TCID50 virus (Wuhan strain; hCoV-19/Japan/TY-WK-521/2020; GISAID ID: EPI_ISL_408667) and serially diluted antibodies (2-fold serial dilutions starting at 1:5 dilution diluted with high glucose DMEM supplemented with 2% FBS and 100 U/mL penicillin/streptomycin) was incubated at 37 °C for 1 hour before being placed on VeroE6/TMPRSS2 cells seeded in 96-well flat-bottom plates. After culturing for 4–6 days at 37 °C supplied with 5% CO₂, cells were fixed with 20%

formalin (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) and stained with crystal violet solution (Sigma-Aldrich, Burlington, MA, USA). Neutralization titer of samples below the detection limit (1:5 dilution) was set as 2.5. We also used First WHO International Standard Anti-SARS-CoV-2 Immunoglobulin (NIBSC code 20/13) to convert our neutralization titer to International Units per milliliter (IU/mL), which showed that a neutralization titer of 113 was equal to 1000 IU/mL (meaning that IU/mL can be estimated if a neutralization titer is multiplied by 8.85).

Data analysis

Data analysis was done using Microsoft Excel, GraphPad Prism, and R. For statistical analysis, Student's t test was used to compare Cq values, Mann-Whitney test was used to compare virus titer, and Kruskal-Wallis test and post-hoc Dunn's test was used to compare antibody titers. P-values of less than 0.05 were considered statistically significant.

Competing interest

The authors declare no conflicts of interest.

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References

1. Coronavirus disease (COVID-19) pandemic. *WHO*
<https://www.who.int/emergencies/diseases/novel-coronavirus-2019> (2021).
2. Polack, F.P. et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N. Engl. J. Med.* **383**, 2603-2615 (2020).
3. Baden, L.R. et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N. Engl. J. Med.* **384**, 403-416 (2021).
4. Voysey, M. et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* **397**, 99-111 (2021).
5. About approval of COVID-19 vaccine under the Pharmaceutical and Medical Device Act (in Japanese). *MHLW* https://www.mhlw.go.jp/stf/newpage_16734.html (2021).
6. Government Chief Information Officers' Portal, Japan: COVID-19 Vaccination Dashboard (in Japanese). National Strategy Office of Information and Communication Technology, Cabinet Secretariat, Government of Japan <https://www.kantei.go.jp/jp/headline/kansensho/vaccine.html> (2021).
7. 41st COVID-19 Advisory Board (June 30, 2021) (in Japanese). *MHLW* https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000121431_00256.html (2021).
8. Harvey, W.T. et al. SARS-CoV-2 variants, spike mutations and immune escape. *Nat. Rev. Microbiol.* **19**, 409-424 (2021).
9. Kustin, T. et al. Evidence for increased breakthrough rates of SARS-CoV-2 variants of concern in BNT162b2-mRNA-vaccinated individuals. *Nat. Med.* <https://doi.org/10.1038/s41591-021-01413-7> (2021).
10. McEwen, A.E. et al. Variants of concern are overrepresented among post-vaccination breakthrough infections of SARS-CoV-2 in Washington State. *Clin. Infect. Dis.* <https://doi.org/10.1093/cid/ciab581> (2021).
11. Greaney, A.J. et al. Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding

- domain that affect recognition by polyclonal human plasma antibodies. *Cell Host Microbe* **29**, 463-476.e6 (2021).
12. Campbell, F. et al. Increased transmissibility and global spread of SARS-CoV-2 variants of concern as at June 2021. *Euro Surveill.* 2021 **26**, 2100509 (2021).
13. Hacısuleyman, E. et al. Vaccine breakthrough infections with SARS-CoV-2 variants. *N. Engl. J. Med.* **384**, 2212-2218 (2021).
14. Levine-Tiefenbrun, M. et al. Initial report of decreased SARS-CoV-2 viral load after inoculation with the BNT162b2 vaccine. *Nat. Med.* **27**, 790-792 (2021).
15. Khoury, D.S. et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* **27**, 1205-1211 (2021).
16. Bergwerk, M. et al. Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. *N. Engl. J. Med.* <https://doi.org/10.1056/NEJMoa2109072> (2021).
17. McMahan, K. et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature* **590**, 630-634 (2021).
18. Active epidemiological investigation of SARS-CoV-2 cases among vaccinated individuals (first report) (in Japanese). *NIID* <https://www.niid.go.jp/niid/ja/diseases/ka/corona-virus/2019-ncov/2488-idsc/iasr-news/10534-498p01.html> (2021).
19. Shirato, K. et al. Development of genetic diagnostic methods for detection for novel coronavirus 2019(nCoV-2019) in Japan. *Jpn. J. Infect. Dis.* **73**, 304-307 (2020).
20. Yamada, S. et al. Assessment of SARS-CoV-2 infectivity of upper respiratory specimens from COVID-19 patients by virus isolation using VeroE6/TMPRSS2 cells. *BMJ Open Respir. Res.* **8**, e000830 (2021).
21. SARS-CoV-2. *ARTIC Network* <https://artic.network/ncov-2019> (2020).
22. Itokawa, K., Sekizuka, T., Hashino, M., Tanaka, R., & Kuroda, M. Disentangling primer interactions improves SARS-CoV-2 genome sequencing by multiplex tiling PCR. *PLoS One* **15**, e0239403 (2020).
23. Evaluation of sensitivity and specificity of four commercially available SARS-CoV-2 antibody

- immunoassays. *PHE*
- https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/898437/Evaluation__of_sensitivity_and_specificity_of_4_commercially_availavai_SARS-CoV-2_antibody_immunoassays.pdf (2020).
24. Evaluation of Roche Elecsys Anti- SARS-CoV-2 serology assay for the detection of anti-SARS-CoV-2 antibodies. *PHE*
- https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/891598/Evaluation_of_Roche_Elecsys_anti_SARS_CoV_2_PHE_200610_v8.1_FINAL.pdf (2020).
25. Evaluation of Roche Elecsys Anti-SARS-CoV-2 S serology assay for the detection of anti-SARS-CoV-2 S antibodies. *PHE*
- https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/989460/Evaluation_of_Roche_Elecsys_anti_SARS_CoV_2_S_assay_PHE.pdf (2021).
26. Salvagno, G.L. et al. Anti-SARS-CoV-2 receptor-binding domain total antibodies response in seropositive and seronegative healthcare workers undergoing COVID-19 mRNA BNT162b2 vaccination. *Diagnostics (Basel)* **11**, 832 (2021).

460 **Table 1.** Demographic and clinical characteristics of COVID-19 breakthrough cases

	All (n=130)	Partially vaccinated (n=63)	Fully vaccinated (n=67)
Age (n (%))			
20-29	37 (28.5)	16 (25.4)	21 (31.3)
30-39	16 (12.3)	4 (6.3)	12 (17.9)
40-49	27 (20.8)	7 (11.1)	20 (29.9)
50-59	14 (10.8)	8 (12.7)	6 (9.0)
60-69	11 (8.5)	6 (9.5)	5 (7.5)
70-79	11 (8.5)	9 (14.3)	2 (3.0)
80+	14 (10.8)	13 (20.6)	1 (1.5)
Sex (n (%))			
Male	37 (28.5)	19 (30.2)	18 (26.9)
Female	93 (71.5)	44 (69.8)	49 (73.1)
Occupation (n (%))			
Healthcare workers	106 (81.5)	42 (66.7)	64 (95.5)
Other	24 (18.5)	21 (33.3)	3 (4.5)
Immunocompromised (n (%); missing=7)			
Yes	0 (0.0)	0 (0.0)	0 (0.0)
No	123 (100.0)	58 (100.0)	65 (100.0)
Immunosuppressant use (n (%); missing=7)			
Yes	3 (2.4)	0 (0.0)	3 (4.5)
No	120 (97.6)	57 (100.0)	63 (95.5)
Vaccine type (n (%); missing=6)			
BNT162b2	121 (97.6)	58 (96.7)	63 (98.4)
mRNA-1273	2 (1.6)	2 (3.3)	0 (0.0)
AZD1222	1 (0.8)	0 (0.0)	1 (1.6)
Vaccination to episode (median (range))			
Days from 1st dose to specimen collection	39 (14-108)	21 (14-64)	59 (35-108)
Days from 1st dose to symptom onset	34 (10-109)	20 (10-41)	61 (32-109)
Days from 2nd dose to specimen collection	23 (0-84)	4 (0-13)	37 (14-84)
Days from 2nd dose to symptom onset	29 (-1-85)	7 (-1-13)	40 (11-85)
Close contact history (n (%); missing=8)			
Yes	92 (75.4)	42 (73.7)	50 (76.9)
No	30 (24.6)	15 (26.3)	15 (23.1)
Severity at the time of reporting* (n (%))			
Asymptomatic	65 (50.0)	29 (46.0)	36 (53.7)
Mild	60 (46.2)	29 (46.0)	31 (46.3)
Moderate	5 (3.8) [†]	5 (7.9)	0 (0.0)
Severe	0 (0.0)	0 (0.0)	0 (0.0)

*Severity is based on Japanese guidance (moderate includes cases with shortness of breath with or without oxygen requirement; severe includes cases intubated and/or cases admitted to the intensive care unit)

[†]Includes 3 requiring oxygen use

Table 2. Viral isolation, mutation-specific PCR, and viral genome analysis of viruses detected in COVID-19 vaccine breakthrough cases

	All	Partially vaccinated	Fully vaccinated
Viral isolation (n=72) (n (%))			
Yes	20 (29.9)	14 (38.9)	6 (19.4)
No	47 (70.1)	22 (61.1)	25 (80.6)
Undeterminable*	5	4	1
Mutation-specific PCR (N501Y) (n=75) (n (%))			
501N	18 (26.5)	10 (27.8)	8 (25.0)
501Y	50 (73.5)	26 (72.2)	24 (75.0)
Under detection limit	7	5	2
Mutation-specific PCR (E484K) (n=75) (n (%))			
484E	63 (90.0)	33 (86.8)	30 (93.8)
484K	7 (10.0)	5 (13.2)	2 (6.3)
Under detection limit	5	3	2
Mutation-specific PCR (L452R) (n=75) (n (%))			
452L	57 (82.6)	31 (83.8)	26 (81.3)
452R	12 (17.4)	6 (16.2)	6 (18.8)
Under detection limit	6	4	2
Viral genome analysis (n=48) (n (%))			
B.1.1.7 (Alpha variant)	34 (70.8)	19 (65.5)	15 (78.9)
B.1.351 (Beta variant)	0 (0.0)	0 (0.0)	0 (0.0)
P.1 (Gamma variant)	1 (2.1)	1 (3.4)	0 (0.0)
B.1.617.2 (Delta variant)	9 (18.8)	5 (17.2)	4 (21.1)
R.1 (includes E484K)	4 (8.3)	4 (13.8)	0 (0.0)

*Cell death possibly due to cytotoxicity of the contaminants in samples

471
472 **Fig. 1. Virological characteristics of breakthrough cases and comparison of lineages with cases**
473 **of SARS-CoV-2 infection submitted to the COVID-19 Genomic Surveillance Network in Japan**
474 **(COG-JP).**

a, quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) done at NIID for all respiratory samples received using NIID-N2 primer/probe set for cases of SARS-CoV-2 infection among partially-vaccinated or fully-vaccinated individuals.

b, viral titer for cases of SARS-CoV-2 infection among partially-vaccinated or fully-vaccinated individuals.

c, correlation between viral RNA and virus titer.

d, f, the number of confirmed lineages based on sequencing and presumed SARS-CoV-2 lineages inferred from RT-qPCR detecting N501Y, E484K, or L452R mutations plotted by week of specimen collection of first positive samples for Eastern Japan (d) and Western Japan (f).

e, g, the number of confirmed lineages in samples submitted to Collaboration with local public health institutes in COG-JP by week of specimen collection for Eastern Japan (e) and Western Japan (g).

Black bars indicate geometric means with 95% confidence interval. ns indicates non-significance. Dotted line indicates a cutoff value.

Fig. 2. SARS-CoV-2 anti-nucleocapsid, anti-spike, and neutralizing antibody response in COVID-19 vaccine breakthrough cases by days since diagnosis and by days since symptom onset

a-c, the levels of anti-nucleocapsid (a), anti-spike (b), and neutralizing (c) antibodies by days since diagnosis (time since collection date of the specimen that first tested positive) for 68 cases (21 acute phase samples and 61 convalescent phase samples with 14 paired samples).

d-f, the levels of anti-nucleocapsid (d), anti-spike (e), and neutralizing (f) antibodies by days since symptom onset for 33 symptomatic cases with known onset date (13 acute phase samples and 29 convalescent phase samples with 9 paired samples).

Green squares denote partially-vaccinated cases while pink circles denote fully-vaccinated cases.

Dotted lines indicate cutoff values.

Fig. 3. SARS-CoV-2 anti-nucleocapsid, anti-spike, and neutralizing antibody response in COVID-19 vaccine breakthrough cases in the acute and convalescent phase

a-c, the levels of anti-nucleocapsid (a), anti-spike (b), and neutralizing (c) antibodies in COVID-19 vaccine breakthrough cases among fully-vaccinated individuals in the acute phase and convalescent phase with two control groups: unvaccinated individuals who have recovered from asymptomatic or mild infection (19-118 days after first PCR positive results) and vaccinated health workers who have never tested positive with PCR and tested negative for anti-nucleocapsid antibodies (24-38 days after second dose).

d-f, the comparison of levels of anti-nucleocapsid (d), anti-spike (e), and neutralizing (f) antibodies

512 between asymptomatic and symptomatic COVID-19 vaccine breakthrough cases.
513 Black bars indicate geometric means with 95% confidence interval. Numbers shown on top are
514 geometric mean titers. Dotted lines indicate cutoff values. Grey horizontal lines in panel e and f
515 indicate geometric mean titer for vaccinated but uninfected individuals as a reference (Post vac.). *
516 denotes statistical significance while ns indicates non-significance.
517

Extended Data Fig. 1. SARS-CoV-2 lineages in COVID-19 vaccine breakthrough cases and in cases with SARS-CoV-2 infection submitted to COG-JP by week of specimen collection.

a, the number of confirmed lineages based on sequencing and presumed SARS-CoV-2 lineages inferred from RT-qPCR detecting N501Y, E484K, or L452R mutations plotted by week of specimen collection of first positive samples for all of Japan.

b, the number of confirmed lineages in samples submitted to COG-JP by week of specimen collection for all of Japan.

Extended Data Fig. 2. SARS-CoV-2 anti-nucleocapsid, anti-spike, and neutralizing antibody response in COVID-19 vaccine breakthrough cases among partially vaccinated individuals in the acute phase and convalescent phase compared with unvaccinated individuals post-infection and vaccinated but uninfected individuals.

a-c, the levels of anti-nucleocapsid (a), anti-spike (b), and neutralizing (c) antibodies in COVID-19 vaccine breakthrough cases among partially vaccinated individuals in the acute phase and convalescent phase with two control groups: unvaccinated individuals who have recovered from asymptomatic or mild infection (19-118 days after first PCR positive results) and vaccinated health workers who have never tested positive with PCR and tested negative for anti-nucleocapsid antibodies (24-38 days after second dose).

Black bars indicate geometric means with 95% confidence interval. Numbers shown on top are geometric mean titers. * denotes statistical significance while ns indicates non-significance. Dotted lines indicate cutoff values.

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