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Note

Neutralising activity and antibody titre in 10 patients with breakthrough infections of the SARS-CoV-2 Omicron variant in Japan

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ABSTRACT

The Omicron variant of severe acute respiratory syndrome coronavirus 2 has multiple amino acid mutations in its spike proteins, which may allow it to evade immunity elicited by vaccination. We examined the neutralising activity and S1-IgG titres in patients with breakthrough infections caused by the Omicron variant after two doses of vaccination. We found that neutralising activity was significantly lower for the Omicron variant than for the Wuhan strain. Two doses of vaccination might not induce sufficient neutralising activity for the Omicron variant.

1. Note

The Omicron variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in South Africa and Botswana in November 2021 [1]. The first case in Japan was reported on November 30, 2021 [2]. This variant has extensive amino acid mutations in its spike proteins, which may allow for the evasion of existing immunity [3]. Here, we measured S1-IgG titres and neutralising activity in patients with breakthrough infections caused by the Omicron variant, then analysed the viral genes in these patients.

Ethical approval was obtained from the institutional ethical review board (approval no. NCGM-G-003472-02). Written consent for participation in the study was obtained from all patients. This retrospective cohort study of breakthrough infection with the SARS-CoV-2 Omicron variant was conducted between November 2021 and January 2022 at the National Centre for Global Health and Medicine (Tokyo, Japan).

The following information was collected from patients' medical charts: demographics and epidemiological characteristics, vaccination status, supportive care and treatment, and outcomes. In total, 10 patients with SARS-CoV-2 Omicron variant breakthrough infection were included. The median age was 40.5 years; nine patients (90.0%) were men. All patients had received vaccines (none had received booster shots): seven had received mRNA-1273 (Moderna) and three had received BNT162b2 (Pfizer-BioNTech). The median time from the second vaccination to onset or diagnosis was 130 days (interquartile range [IQR]: 117–134). Two patients were asymptomatic, while eight patients had mild disease that did not require oxygen supplementation. One patient received sotrovimab (No. 9); no patients received corticosteroids or died (Supplementary Table S1).

SARS-CoV-2 whole genome sequencing was performed using nasopharyngeal swab samples to confirm the presence of the Omicron variant (GISAID ID: EPI_ISL_6913953, 6914908, 7194610, 7860185, 7860188, 7860189, 7860190, 8753745, 8753746, and 8753747). All 10 SARS-CoV-2 genomes were classified as Omicron, of which seven were BA.1 lineage and three were BA.1.1 lineage. The spike proteins of all Omicron variants in this study had at least 30 amino acid substitutions, three deletions, and one insertion, including G142D in the N-terminal domain (NTD); K417N, E484A, N501Y, and D614G in the receptor-binding domain (RBD). Additionally, T76I substitution was found in one BA.1 lineage, and all three BA.1.1 lineage (a sublineage of BA.1)

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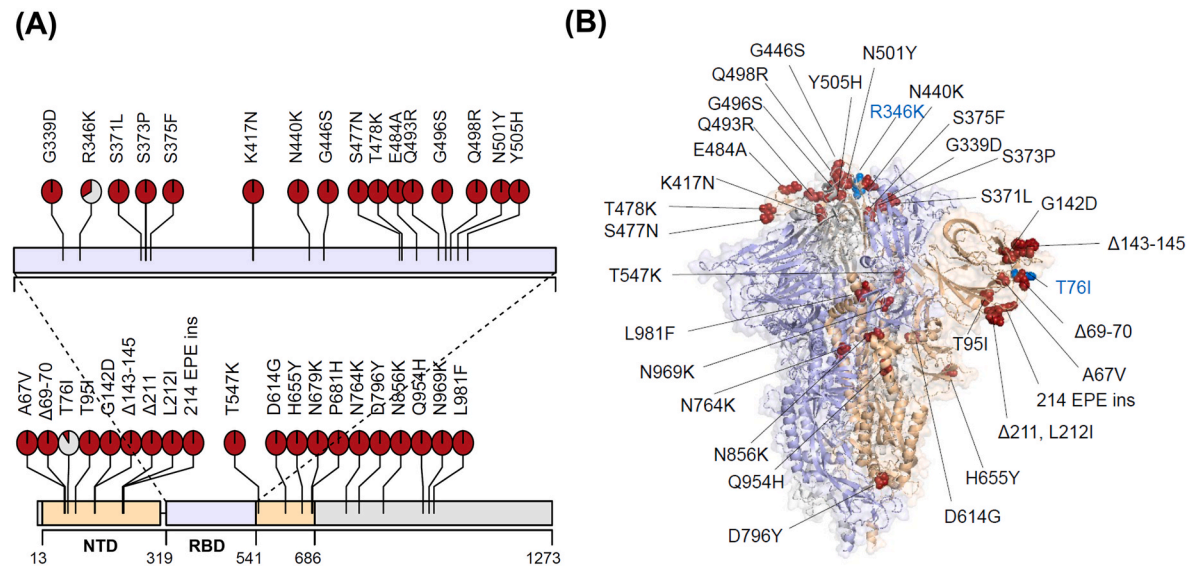

Fig. 1. Spike mutations observed in 10 patient-derived SARS-CoV-2 Omicron variants. (A) (left) The coloured part of the pie chart corresponds to the percentage of patients with each mutation. The schematic representation was generated using the LolliPlot function of the trackVignette package in R [4]. NTD, N-terminal domain; RBD, receptor-binding domain. (B) (right) Locations of spike amino acid mutations observed in this study. The cryo-EM structure of the SARS-CoV-2 trimeric spike glycoprotein (PDB code: 7DF3) [5] was generated using PyMOL v2.5.2. Three protomers are shown in different colours. Red and blue dots represent the mutated residues observed in all or some patients in the present study, respectively.

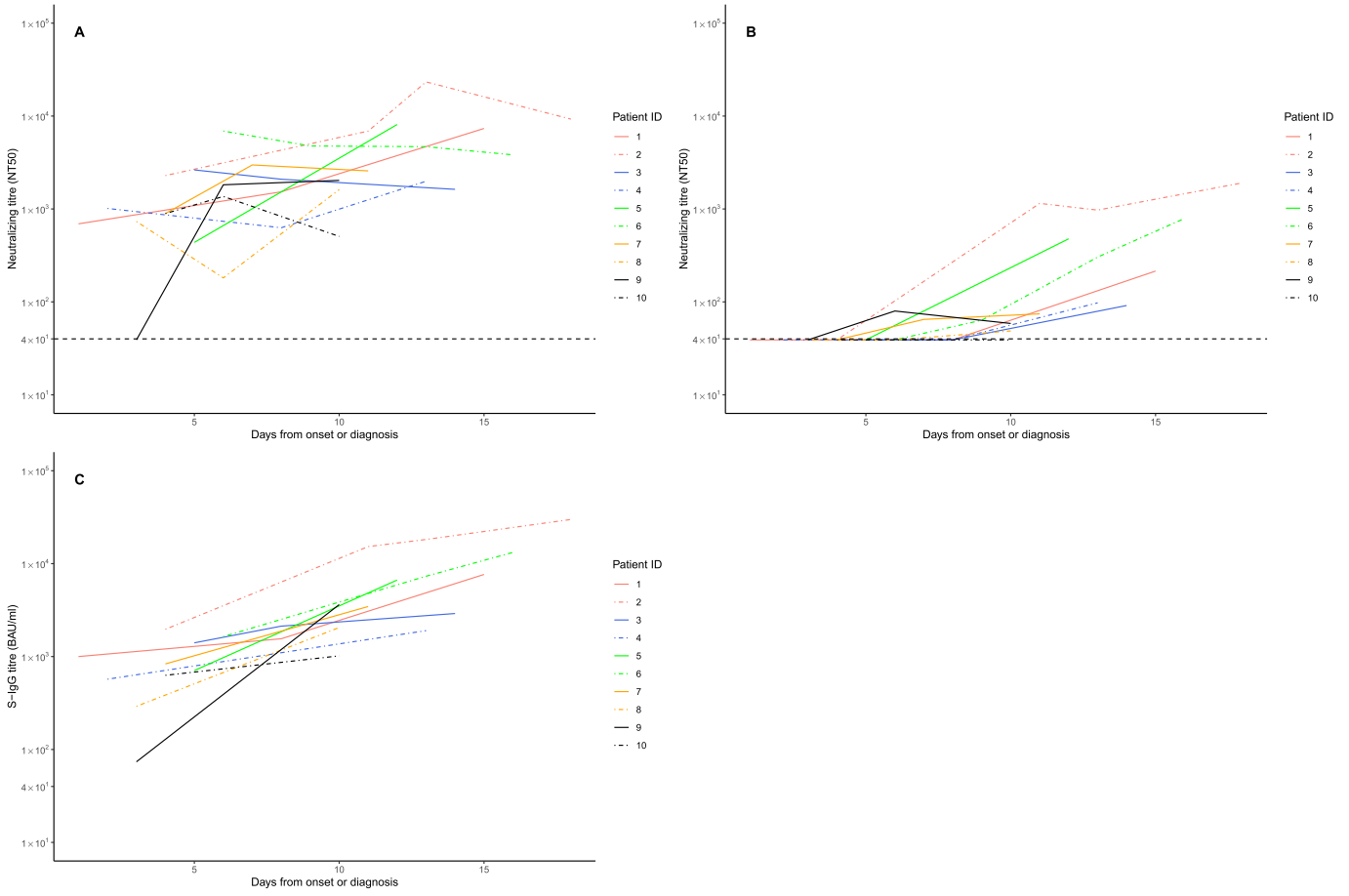

Fig. 2. Kinetics of the neutralising activity of the ancestral lineage and the Omicron variant and the S1-IgG titres. (A) Neutralising activities are shown as the 50% neutralising titre (NT_{50}) for WK-521 (Wuhan). (B) Neutralising activities are shown as the 50% neutralising titre (NT_{50}) for 929-1 N (Omicron). An NT_{50} value of 40 is the detection limit and the values < 40 were regarded as 40. All assays were performed in duplicate. (C) S1-IgG titres.

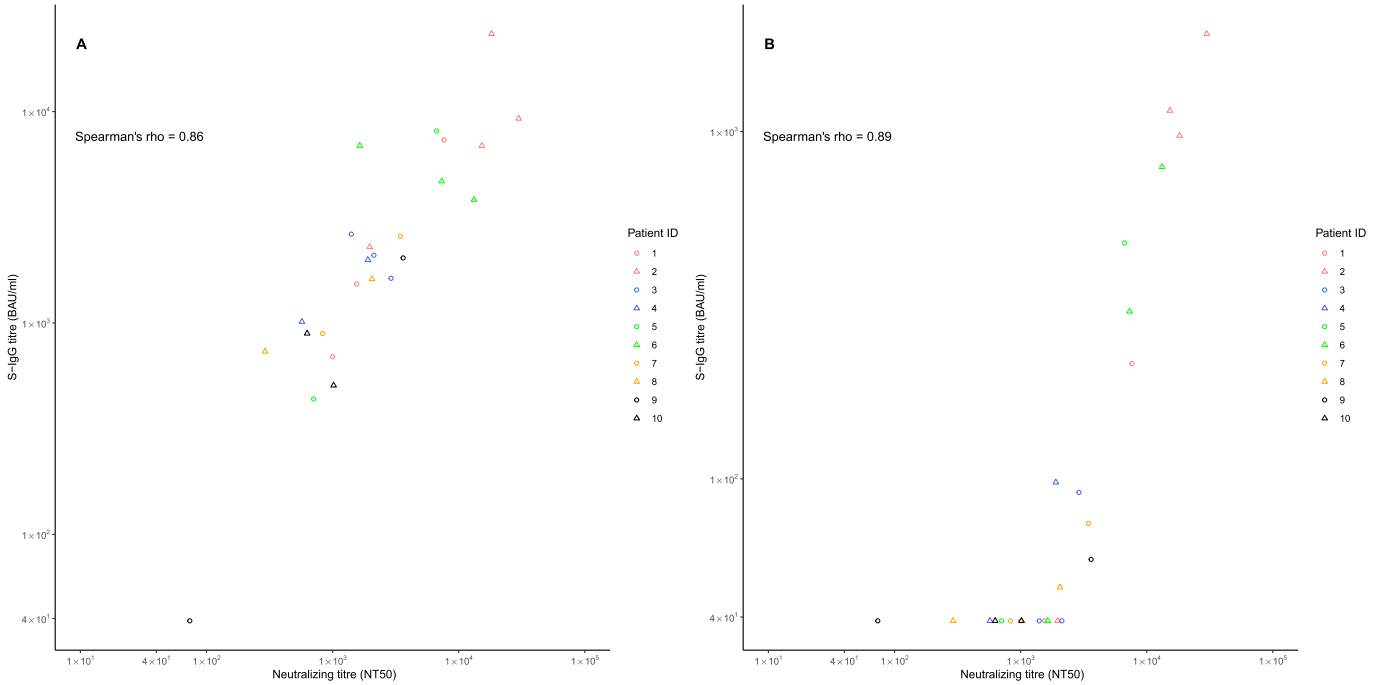

Fig. 3. Correlation between the SARS-CoV-2 S1-IgG titres and neutralising activity. (A) Correlation between the S1-IgG titres and neutralising titre (NT_{50}) for WK-521 (Wuhan). (B) Correlation between the S1-IgG titres and neutralising titre (NT_{50}) for 929-1 N (Omicron).

possessed R346K substitution (Fig. 1).

The levels of anti-SARS-CoV-2 S1-IgG antibodies were determined using the HISCL anti-SARS-CoV-2 immunoassay (Sysmex, Kobe, Japan), as previously described [6]. Each SARS-CoV-2 strain (2019-nCoV/Japan/TY/WK-521/2020 [Wuhan; ancestral lineage] and SARS-CoV-2/NCGM929-1 N [Omicron]) isolated from hCoV-19/Japan/IC-2279/2021 was propagated in VeroE6/TMPRSS2 cells. For the neutralisation assay, each virus was added to serially-diluted sera/plasma, incubated for 20 min at 37 °C, and used to inoculate HeLa-ACE2-TMPRSS2 cells. After 3–4 days, the cytopathic effect in the culture was assessed [7]. The 50% neutralising titre (NT_{50}) values for the ancestral lineage and the Omicron variant were measured using each sample.

We compared NT_{50} values for the ancestral lineage and the Omicron variant using the Wilcoxon signed-rank test, using the first samples from each patient. We also examined correlations between the S1-IgG titres and NT_{50} values for both variants, using the Spearman rank correlation coefficient. NT_{50} values lower than 40 were regarded as 39 in statistical analysis.

In total, 25 and 31 blood samples were obtained from 10 patients and analysed for S1-IgG titres and NT_{50} values, respectively. In the initial samples collected at a median of 4 days (IQR: 3–5) after disease onset or diagnosis, NT_{50} values were detectable for the ancestral lineage; however, no samples showed detectable neutralisation of the Omicron variant (Fig. 2). NT_{50} values of the initial samples were significantly lower for the Omicron variant than for the ancestral lineage ($p < 0.001$).

Additionally, the median S1-IgG titre was 770.2 binding antibody units (BAU)/ml (IQR: 585.0–1303.8) in the first samples; this titre was 3539.0 BAU/ml (IQR: 2266.0–7400.0) in the last samples collected at a median of 12.5 (median, IQR: 10–15) days after disease onset or diagnosis (Fig. 3). Strong correlations were identified between NT_{50} values for the ancestral lineage and S1-IgG titres, and between NT_{50} values for the Omicron variant and S1-IgG titres (Spearman $\rho = 0.86$ and 0.89, respectively).

The results of this study confirmed that neutralising activity against the Omicron variant was undetectable and the NT_{50} values obtained were significantly lower than against the ancestral lineage immediately after diagnosis. This suggests that breakthrough infection of the Omicron variant is caused by immunity attenuation after vaccination; additionally, immunity elicited by two doses of BNT162b2 or mRNA-1273 may not be completely effective for preventing Omicron variant infection. Several other studies have reported similar findings. In one study using plasma from BNT162b2-vaccinated participants, the ability to neutralize the Omicron variant was lower than for the ancestral lineage. The geometric mean titre of the focus reduction neutralisation test value showed a 22-fold decline [8]. Another study showed that the 50% neutralisation titre for the Omicron variant in plasma from persons administered two doses of an mRNA vaccine (BNT162b2 or mRNA-1273) at 1 month and 5 months after vaccination was 127 and 27 times lower than that for the ancestral lineage, respectively [9]. These results can be explained by the fact that the Omicron variant has multiple amino acid mutations in the spike protein. Among the mutations detected in our patients, G142D, N501Y, and D614G are reportedly associated with breakthrough infection [10]. The K417N and E484A mutations, detected in all 10 patients, have been shown *in silico* to reduce vaccine efficacy in a complementary manner [11]. We should be aware that the evaluation of each mutation alone may not necessarily reflect the characteristics of the virus as a whole because escape from immunity depends on the combination of mutations each virus possesses. In one study, a synthetic polymutant spike protein with approximately 20 amino acid mutations, some of which were shared by the Omicron variant, showed substantial evasion of the polyclonal neutralising antibodies elicited in persons who have recovered from COVID-19 or have received two doses of an mRNA vaccine [12]. Our findings indicate that breakthrough infections can occur in previously vaccinated individuals because the Omicron variant carries multiple amino acid mutations associated with immune evasion.

Furthermore, NT_{50} values for the Omicron variant increased after infection had occurred (i.e., between the first and last samples) in nine patients. The elevation of neutralising activity was observed, at the earliest, 3 days after onset or diagnosis. One patient showed no increase of neutralising activity against Omicron after infection, suggesting that a certain percentage of people infected with the Omicron variant do not seroconvert, as observed for those infected with the ancestral lineage

[13]. The NT_{50} values for the ancestral lineage also increased in a similar manner in seven patients. The NT_{50} values considerably increased in one patient who had low neutralising activity immediately after diagnosis. Our results suggest that infection with the Omicron variant could induce cross-neutralising activity to the ancestral lineage, particularly in patients with low neutralising activity prior to infection.

The median time from the second dose of mRNA vaccine to diagnosis or onset in patients in this study was 130 days. After diagnosis or onset, S1-IgG titres increased in all patients. In a previous study, S1-IgG titres reached a peak 28 days after the first dose (i.e., 7 days after the second dose) of BNT162b2 and declined linearly thereafter [14]; thus, S1-IgG titres in our patients might have decreased over time but recovered upon infection with the Omicron variant.

Our results also indicated that a higher S1-IgG titre was associated with higher NT_{50} values for both the ancestral lineage and the Omicron variant. This correlation is consistent with previous findings concerning positive correlations of neutralising activity with S- or S1-IgG titres after vaccination [14] or infection [13]. While there are only a limited number of facilities that can analyse neutralising activity, we might predict neutralising activity by measuring S1-IgG titres.

The limitation of our study is that a smaller number of patients (all returnees from travel abroad) were analysed than in a previous study [9]. Although the smaller sample size may affect the reliability of the correlation between S1-IgG titres and NT_{50} values, our study showed a high correlation coefficient.

In this study of 10 patients with breakthrough infections of the Omicron variant in Japan, we found that initial samples collected after disease onset or diagnosis exhibited significantly lower neutralising activity for the Omicron variant than for the ancestral lineage. Notably, no samples showed detectable neutralisation for the Omicron variant. Two doses of vaccination might not induce sufficient neutralising activity for the Omicron variant in this study time course.

Authors' contributions

NOkumura, ST, SS, NI, and NOhmagari designed the study. NOkumura, ST, SS, MU, MH, and NI implemented the study and collected data. SH determined the neutralising activity. JT and TS were responsible for the mutational analysis. NOkumura, ST, and SS wrote the first draft of the manuscript. WS, HM, and NOhmagari supervised this study. All authors revised the manuscript and approved the final version.

Declaration of competing interest

The authors declare no conflicts of interest associated with this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jiac.2022.04.018.

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