Comparison of the clinical features, viral shedding and immune response in vaccine breakthrough infection by the Omicron and Delta variants

Barnaby Young (✉ barnaby_young@ncid.sg)

National Centre for Infectious Diseases <https://orcid.org/0000-0003-1010-2230>

Siew-Wai Fong

A*STAR Infectious Disease Labs (A*STAR ID Labs), Agency for Science, Technology and Research

Zi Wei Chang

A*STAR Infectious Disease Labs (A*STAR ID Labs), Agency for Science, Technology and Research

Kai Sen Tan

National University of Singapore

Angeline Rouers

A*STAR Infectious Disease Labs (A*STAR ID Labs), Agency for Science, Technology and Research

Yun Shan Goh

A*STAR Infectious Disease Labs (A*STAR ID Labs), Agency for Science, Technology and Research

Douglas Jie Wen Tay

National University of Singapore

Sean W. X. Ong

MOHH <https://orcid.org/0000-0002-8570-436X>

Ying Hao

National Centre for Infectious Diseases

Siang Li Chua

National Centre for Infectious Diseases

Jean-Marc Chavatte

National Centre for Infectious Diseases <https://orcid.org/0000-0002-0003-541X>

Lin Cui

National Centre for Infectious Diseases

Matthew Tay

A*STAR ID Labs, A*STAR

Raymond Tzer Pin Lin

National Centre for Infectious Diseases

Laurent Renia

A*STAR Infectious Disease Labs (A*STAR ID Labs), Agency for Science, Technology and Research

Yee-Sin Leo

National Centre for Infectious Diseases <https://orcid.org/0000-0003-4978-5825>

Justin Jang Hann Chu

National University of Singapore <https://orcid.org/0000-0002-1673-6819>

David Lye

National Centre for Infectious Diseases

Lisa Fong Poh Ng

A*STAR Infectious Disease Labs (A*STAR ID Labs), Agency for Science, Technology and Research

<https://orcid.org/0000-0003-4071-5222>

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Abstract

Background

On 26 November 2021, the World Health Organization designated the B.1.1.529 lineage of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) as the fifth variant of concern, Omicron. Infections have quickly spread worldwide, but understanding of the viral dynamics and the cytokine and cellular immunological response during infection remain limited.

Methods

Detailed patient-level data from 174 age-matched patients with sequence confirmed Omicron or Delta infection admitted to the National Centre for Infectious Diseases, Singapore were analyzed in an observational cohort study. Peripheral blood samples for measurement of SARS-CoV-2 immunological parameters were obtained from a subset. Respiratory samples were collected for viral cultures and correlated to corresponding PCR cycle threshold (Ct) values.

Results

Omicron and Delta variant infections in this hospitalized cohort were mild with only 3 (3%) and 14 (16%) developing pneumonia respectively. Omicron infections were more likely to present with sore throat (46.0 vs 23.0%, $p=0.005$). Neutrophil counts and C-reactive protein (CRP) were significantly lower among the Omicron cohort (Median neutrophil 2.95 [IQR 2.16 – 3.96] vs 4.60 [IQR 3.76 – 6.10] $\times 10^9/L$, $p<0.001$; Median CRP 5.7 [IQR 2.0 – 10.0] vs 12.0 [IQR 6.1 – 22.0] mg/L, $p<0.001$). Trough polymerase chain reaction (PCR) cycle threshold (Ct) values were significantly higher with Omicron infection (17.6 [IQR 16.3 – 19.3] vs 14.9 [IQR 13.9 – 19.0], $p=0.001$). The pattern and rate of rise in Ct values was similar between Omicron and Delta. At the time of infection, Omicron infected patients had lower levels of pro-inflammatory cytokines. Vaccine breakthrough infections with the Omicron variant had a low concentration of proinflammatory cytokines, chemokines, and growth factors at the acute phase of infection, but a more robust IFN- γ response. Less dysregulated immune cell profiles were also observed, including a lower immature neutrophil cell count in Omicron breakthrough cases.

Conclusions

Omicron infections resulted in mild vaccine breakthrough illness in the majority of patients. Compared with Delta, Omicron infections were more frequently associated with upper respiratory tract infections, had lower viral loads, lower levels of pro-inflammatory cytokines and less dysregulated immune cell profiles.

Introduction

Since the emergence of the Alpha (B.1.1.7) variant in September 2020, the Coronavirus disease 2019 (COVID-19) pandemic has been shaped by successive waves of variants with increased transmissibility

and/or immune evasion compared with the ancestral strain.¹ Delta (B.1.617.2) caused a large outbreak in India from February 2021 and displaced other variants to become globally dominant over the following six months.² Omicron (B.1.1.529) was first reported from Gauteng province, South Africa in November 2021.³ On 26 November 2021 it was classified as the fifth variant of concern (VOC) by the World Health Organization (WHO) and has spread rapidly to many countries worldwide.

The Omicron variant is characterized by >30 mutations in the gene encoding for the spike protein.⁴ Some of the deletions and mutations identified in the Omicron variant (e.g., 69-70del, T95I, G142D/143-145del) are known to result in higher viral binding affinity, higher rates of antibody escape and increased transmissibility.^{5,6} As a result of these changes, Omicron is able to avoid neutralization by serum samples from recovered or vaccinated individuals and by a large range of human monoclonal antibodies.⁷⁻¹⁰

Mice experimentally infected with Omicron had less severe clinical signs (less weight loss and severe pneumonia) and lower viral loads in lower and upper respiratory tract compared with Delta.¹¹ Lower viral loads and no histopathological evidence of bronchopulmonary inflammation were found in Omicron infected hamsters.¹² In a preliminary report, Ferguson et al. recently described a reduction in risk of hospitalization for Omicron relative to Delta, including an apparent difference by vaccination state.¹³ However, immunological and clinical data remain limited.

In December 2021, Singapore saw a wave of imported Omicron infections with limited local transmission. As part of the initial public health response all individuals with suspected Omicron infection (S-gene target failure) were admitted to the National Centre for Infectious Diseases (NCID) for isolation. Close contacts were identified, isolated in a dedicated facility for 10 days, with PCR testing at the start and end of the quarantine period.

A more granular understanding of viral shedding, as well as inflammatory mediators and cellular immune features of emerging VOCs is essential in guiding healthcare policies globally, including isolation, hospitalization, and vaccination policies. In this cohort study we characterize the clinical features, virological and immunological kinetics of patients with vaccine breakthrough COVID-19 caused by the Omicron variant in comparison with Delta.

Methods

Patient Recruitment

Individuals confirmed to have COVID-19 by SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction (RT-PCR), admitted to the National Centre for Infectious Diseases (NCID), Singapore, and fully vaccinated were eligible for inclusion in the study. Fully vaccinated was defined as infection onset ≥ 14 days after completion of any primary COVID-19 vaccine series with a WHO Emergency Use Listing (EUL).

Illness onset was defined as whichever was early from date of symptom onset or first PCR with SARS-CoV-2 detected.

Two age-matched (± 10 years) cohorts were selected: Omicron (B.1.1529) or Delta (B.1.617.2) variant infections as assigned by direct whole-genome sequencing (WGS) of RNA extracted from nasopharyngeal swabs by the National Public Health Laboratory (NPHL) at NCID. Pangolin COVID-19 Lineage Assigner and CoVsurver were used to assign sequence lineages. Individuals were included if they were admitted to NCID between 1-18 Dec 2021 for Omicron and 27 April to 11 Aug 2021 for Delta. During these two periods, all individuals with Omicron or Delta infection respectively were admitted to hospital for isolation and evaluation regardless of disease severity, and NPHL attempted WGS for all infections with RT-PCR Cycle threshold (Ct) value <30 .

Clinical Chart Review and Sample Collection

Clinical information was extracted from the medical record using a standardized data collection form adapted from the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) case record form as previously described.¹⁴ Laboratory data including baseline investigations, Ct values from RT-PCR and serologic tests were recorded. Serial blood and respiratory samples were collected during hospitalization as part of clinical care. Serological results from Elecsys® (Roche, Basel, Switzerland) Anti-SARS-CoV-2 chemiluminescent immunoassays [anti-nucleocapsid (anti-N) and anti-spike protein receptor binding domain (anti-S)]. SARS-CoV-2 PCR was performed with a range of commercially available assays used by the clinical laboratory. Repeat RT-PCR testing was conducted at intervals based on institutional protocol and as decided by the managing physicians. At the time of the study, patients were de-isolated based on discharge criteria of at least 10 days from illness onset with Ct values ≥ 30 on RT-PCR of nasopharyngeal swabs.

Ethics Statement and Data Availability

Waiver of informed consent for collection of clinical data from individuals infected with the Omicron variant was granted by the Ministry of Health (MOH), Singapore, under the Infectious Diseases Act as part of the COVID-19 outbreak investigation. Retrospective data collection from individuals with Delta infection was approved by the institutional ethics committee (REF: 2020/01122). Written informed consent was obtained from study participants for collection of biological samples after review (REF DSRB: 2012/00917). All data sharing requests should be addressed to the corresponding authors.

Virus Isolation

Four hundred μ l of the processed and filtered supernatant of nasopharyngeal swab obtained from SARS-CoV-2-positive individuals was inoculated into Vero E6 TMPRSS2 cells¹⁵ in a biosafety level 3 laboratory. Cells were cultured at 37 °C under 5% CO₂ for two rounds of passages (with the second passage performed at 3 days post infection of the first passage).^{16,17} The cytopathic effect (CPE) was observed on day 6 post infection for each passage under an inverted microscope. Positive isolation was confirmed

by observation of CPE and positive RT-PCR of SARS-CoV-2 N2 gene (CDC N2). At the end of second passage, positive CPE cultures were extracted for viral RNA to perform PCR to confirm if isolated virus was from Omicron infection using Omicron specific S-gene primers (F: 5'-TACAACTTGTGCCCTTTTGA-3'; R: 5'-AAAAGTGAAAAATGGTGCGAG-3').

Multiplex microbead-based immunoassay

Plasma samples were inactivated with Triton™ X-100 (Thermo Fisher Scientific) to a final concentration of 1% for two hours in the dark.^{18,19} Measurement of immune mediators was done using the Cytokine/Chemokine/Growth Factor 45-plex Human ProcartaPlex™ (Thermo Fisher Scientific) with the Luminex™ assay (Supplementary Appendix 1). Immune mediator levels were measured in 17 vaccinated healthy donor plasma as baseline controls. The value of Limit of Quantification (LOQ) was assigned to samples with concentrations out of the measurement range.

Whole blood immunophenotyping

Twenty five µL of whole blood was stained with antibodies as stated in Supplementary Table 1 for 20 min in the dark at room temperature (RT) and followed by lysis of erythrocytes with 1 mL of 1.2X BD FACS Lysing solution (BD cat#349202) for 10 min at RT. 300 µL of PBS was added after the incubation and centrifugated at 800 x g for 5 min. Samples were then washed with 1 mL of PBS and transferred to polystyrene FACS tubes containing 5 mL (2.4×10^3 beads) of CountBright Absolute Counting Beads (Invitrogen Cat#C36950, Lot 2324193). Cells were acquired with the Cytek™ Aurora cytometer running SpectroFlo® Version 2.2.0.3 with automated unmixing and analyzed using FlowJo v10.8.0.

SARS-CoV-2-specific T cells by Intracellular Cytokine Staining

To profile the SARS-CoV-2 specific T effector subsets in COVID-19 patients, intracellular cytokine staining was carried out as previously described²⁰ with modification. Briefly, PBMCs collected from whole blood of vaccine breakthrough cases and healthy vaccinated controls were stimulated with phorbol 12-myristate 13-acetate (PMA 100ng/mL, Sigma Aldrich) and ionomycin (1µg/mL, Sigma Aldrich), or pooled SARS-CoV-2 PepTivator S, S1, M and N peptides (0.6nmol/mL each) (Miltenyi Biotec) for six hours. Brefeldin A and Monesin (1×, ThermoFisher Scientific) were added at two hours post-stimulation. Cells were stained for surface markers in the dark at room temperature for 30 minutes (Supplemental Table 2, Row 1-21), followed by fixation and permeabilization for 30 mins with Foxp3/Transcription Factor Staining Buffer Set (ThermoFisher Scientific). Permeabilized cells were then stained for intracellular cytokines in the dark at room temperature for 30 minutes (Supplemental Table 2, Row 22-29). Cells were then washed with PBS and centrifuged at 800 x g for 5 minutes before transferring to respective polystyrene FACS tubes containing 5 mL (4.8×10^3 beads) of CountBright Absolute Counting Beads (Invitrogen Cat#C36950, Lot 2324193). Cells were acquired with the Cytek™ Aurora cytometer running SpectroFlo® Version 2.2.0.3 with automated unmixing and analyzed using FlowJo v10.8.0.

Spike protein flow cytometry-based assay (SFB assay) for antibody detection

The SFB assay was performed as previously described.^{21,22} S protein-expressing cells were seeded at 1.5×10^5 cells per well in 96 well V-bottom plates. Cells were incubated with human serum (diluted 1:100 in 10% FBS) followed by a secondary incubation with a double stain, comprising Alexa Fluor 647-conjugated anti-human IgG (1:500 dilution) and propidium iodide (PI; 1:2500 dilution). Cells were acquired using a BD Biosciences LSR4 laser and analyzed using FlowJo (Tree Star). The assay was performed as two independent experiments with technical duplicates each time.

Memory B cell ELISpot

SARS-CoV-2 RBD-specific memory B cell numbers were counted using ELISpot. MultiScreenHTS IP Filter Plate, 0.45 μ m plates (Merck Millipore) were coated overnight at 4°C with purified anti-human-IgG (MT91/145, Mabtech) prepared at 15 μ g/ml in PBS 1X. Plates were washed 4 times with PBS 1X and blocked at least 30 minutes at room temperature with RPMI 10% FBS. Fresh PBMCs were plated directly into ELISpot plate (100,000 and 400,000) to determine RBD-specific plasmablast numbers. In parallel, 1×10^6 PBMCs were resuspended in 1ml RPMI + 10% FBS + 1 μ g/ml R848 + 10 ng/ml IL-2, and incubated at 37°C, 5% CO₂ for 4-5 days to differentiate memory B cells into antibody-secreting cells. After incubation, cells were counted, and 100,000 or 400,000 live cells were taken for ELISpot plating to determine RBD-specific memory B cell numbers. Total IgG secreting cells were determined by plating 1,500 or 3,000 live cells. In both cases (plasmablasts or memory B cells), cells were incubated 18-22h at 37°C, 5% CO₂ in the ELISpot plate before detection. A combination of RBD-WASP/anti-WASP-ALP or anti-IgG-biotinylated/streptavidin-ALP (Mabtech) were used to detect RBD-specific or total IgG secreting cells respectively. Plates were then read on an IRIS ELISpot reader (Mabtech). Spots were calculated based on the average of two wells.

Statistical Analysis

For descriptive analysis, data are presented as median (interquartile range [IQR]) for continuous parameters and frequency (percentage) for categorical variables. Fisher's exact test was used to compare categorical variables, while for continuous variables, t-test was used for normal data and Mann-Whitney U test for non-normal data. All statistical tests were 2-sided and *P* values <0.05 were considered statistically significant. For asymptomatic patients, day one of illness was assigned to the day of confirmatory diagnosis by RT-PCR. For symptomatic patients, day of symptom onset was denoted as day one of illness.

For serial Ct values, we fitted a generalized additive mixed model (GAMM) with a random intercept by patient. A Ct value of 45 was imputed where the PCR result was not detected. We compared this with previously reported serial Ct values of Delta infected patients.²³ We plotted Ct values with marginal effect of day of illness by variant type and 95% confidence intervals (CI) from the GAMM.

For immunological analysis, to compare between multiple groups, Kruskal-Wallis tests with post hoc tests using Dunn's multiple comparison tests were used to identify significant differences. For whole blood

immunophenotyping, gated cells were manually exported using FlowJo v10.8.0. Samples were then used for Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP) analysis using cytofit2 R Packages with RStudio v3.5.2.²⁴ Thirteen healthy vaccinated controls, 15 Delta vaccine breakthrough patients and 17 Omicron breakthrough patients were each concatenated to their respective groups and 150,000 cells were analyzed using the ceil method. Principal Component Analysis (PCA) was performed on the systemic cytokine data using Singular Value Decomposition (SVD) method in ClustVis.²⁵

Results

Clinical Features

Of the 101 patients diagnosed with Omicron vaccine breakthrough, 87 (87%) were matched with the cohort of 287 Delta vaccine breakthrough infections (Supplementary Figure 1). The Omicron cohort was younger (Median age 34 [IQR 29- 43] vs 43 [IQR 33 – 52] years, $p<0.001$), but had similar sex and comorbidity profile (Table 1). Omicron infected patients were more likely to report symptoms of an upper respiratory infection (sore throat 46.0 vs 23.0 %, $p=0.005$) and less likely to develop pneumonia (3.4 vs 16.1%, $p=0.005$). Median neutrophil count, C-reactive protein and lactate dehydrogenase levels were lower in Omicron infections.

Table 2 compares Omicron infected patients with two doses of vaccination and booster vaccination. Patients with booster vaccination were significantly older and had higher anti-Spike antibody but were similar in clinical and laboratory features including median initial and lowest PCR cycle threshold values. All 3 cases of re-infection had not received a booster dose.

Virologic kinetics

Serial Ct values of individual patients were analyzed as a surrogate marker for the viral load. Ct value at presentation was significantly higher with Omicron compared with Delta infections (20.7 [IQR 17.9 – 28.5] vs. 19.1 [15.4 – 21.1], $p<0.001$). Pattern of viral shedding was comparable for Omicron and Delta, with an increase in viral load over the first 2-3 days of illness, and significant decline from Day 8 (Figure 1). Trough and illness onset median Ct values were similar between regardless of administration of a vaccine booster vaccination doses (Table 2).

Viral cultures were attempted from 22 Omicron respiratory samples where SARS-CoV-2 was detected by RT-PCR, collected from 14 patients. Negative viral cultures were obtained starting from day 2 of illness. There were no positive viral cultures for patients beyond day 5 of illness or with Ct values > 26 (Supplementary Figure 2).

Serologic Data

Serologic data was available on record for 81 individuals. Median anti-SARS-CoV-2 anti-Spike protein (anti-S) antibody titers in the 81 patients was 1,841 U/mL (IQR 1,060.8 – 5,880). Patients who received >2 vaccination doses had higher median anti-S antibody titers ($P < 0.001$).

The Omicron breakthrough cases had high anti-SARS-CoV-2 S antigen SFB binding, with median IgG SFB binding at 39.45% (IQR 31.3 – 52.84%). Patients who received >2 vaccination doses had higher median IgG SFB binding, but otherwise there was no significant difference in IgG SFB responses between Omicron breakthrough cases and vaccinated healthy controls or Delta breakthrough infections (Supplementary Figure 3).

Systemic cytokine response

We assessed the immune soluble mediators in acute plasma samples from a subset of Omicron breakthrough cases ($n=17$) with a 45-plex microbead-based immunoassay (ThermoFisher) and compared their systemic cytokine responses with age-matched vaccinated healthy controls and Delta breakthrough infections. Omicron breakthrough cases had systemic cytokine profiles similar to their age-matched Delta breakthrough infections. Both Omicron and Delta breakthrough infections showed cytokine profiles that clustered closely to their age-matched vaccinated healthy controls (Figure 2a). Levels of previously identified severity-associated cytokines¹⁴, HGF, IL-12p70, IL-1RA, IL-6, MIP-1a, PDGF-BB and VEGF-A were not significantly elevated in the Omicron breakthrough infections compared with vaccinated healthy controls (Figure 2b). Nevertheless, these patients had higher concentrations of IP-10 (also called CXCL10), an inflammatory marker induced by IFN-gamma²⁶, compared with healthy vaccinated controls (Figure 2b). In addition, Omicron breakthrough has a lower level of proinflammatory VEGF-A than the Delta infection.

Whole blood immunophenotyping

Unbiased dimensionality reduction and clustering analysis by Uniform Manifold Approximation and Projection (UMAP) identified three immune cell clusters, T cells, natural killer cells and neutrophils, with variation in Omicron breakthrough cases compared to vaccinated healthy controls and Delta vaccine breakthrough infections (Figure 3a). Further analysis revealed a reduced cell count for CD4 and $\gamma\delta 1$ T cells (Figure 4b), and an increased cell count for total natural killer (NK) cells and CD56 dim NK cells in patients with acute Omicron breakthrough infection than vaccinated healthy controls (Supplemental Figure 4-6). No significant changes were observed for dendritic cells, total monocytes and eosinophils (Figure 3b) between Omicron breakthrough cases and vaccinated healthy controls. In comparison to Delta infections, Omicron infections had higher counts of intermediate monocytes, dendritic cells and NK cells, and a lower count of $\gamma\delta 1$ T cells. There was a difference in the composition of neutrophil subsets between Omicron and Delta breakthrough infections, where a higher level of mature neutrophils accompanied a lower immature neutrophil cell count in Omicron breakthrough cases (Figure 3b). Together, our data suggest milder alterations in the immune cell compartments during Omicron

breakthrough infections when compared to vaccinated healthy controls and Delta vaccine breakthrough infections.

Memory B and T cell responses

To further define their immunological status at the time of breakthrough infection, we looked at the levels of memory B and T cell responses in these Omicron patients. Using a B cell ELISpot targeting the SARS-CoV-2 receptor-binding region, we examined the frequency of SARS-CoV-2-specific memory B cells. There was no significant difference between Omicron and vaccinated healthy controls in the frequency of RBD-specific memory B cells (Figure 4a), but we observed a significantly higher frequency of RBD-specific memory B cells in Omicron cases compared with Delta cases. The antibody-secreting plasmablast responses were much higher in vaccine breakthrough cases than vaccinated healthy controls, with no significant difference between Delta and Omicron infections (Figure 4a). SARS-CoV-2-specific T cell responses were also analyzed in these Omicron breakthrough patients. PBMCs were stimulated with a pool of SARS-CoV-2-derived epitope peptides, followed by intracellular staining for cytokines. Although there was no significant difference in CD4 Th₁, Th₂, Th₁₇, and CD8 responses between Omicron breakthrough cases and vaccinated healthy controls, Omicron patients had lower frequencies of CD4+ TNF-α+ cells (Figure 4b, Supplemental Figure 7).

Discussion

Since the beginning of January 2022, Singapore has reported an increasing number of locally acquired COVID-19 infections, of which the majority are now due to the Omicron variant.²⁷ At the time of data acquisition for this study, all suspected Omicron infected patients (S gene target failure detected) were hospitalized at NCID and as such, our cohort captures the full spectrum of disease severity. In our study the majority of patients with Omicron infection had mild illness with upper respiratory tract infections similar to reports elsewhere.²⁸ We also found a lower proportion of Omicron infected patients with pneumonia compared with Delta in an age-matched cohort.

The Imperial College COVID-19 response team estimated a 15-20% reduction in hospitalization compared with those infected with Delta¹³. In our study population, the majority of patients did not require hospitalization based on severity of illness. Mild illness partially reflects the population affected in this cohort – younger patients with few comorbidities; most were returning travelers. This may also be related to the predilection of Omicron for upper versus lower respiratory tract.²⁹ COVID-19 vaccination is associated with more asymptomatic infections and better clinical outcomes with Delta breakthrough infection.³⁰ We were unable to determine the consequences of Omicron infection in unvaccinated individuals.

Although few studies had reported reduced neutralization of Omicron post-vaccination^{7,8}, Dejnirattisai et al.⁹ demonstrated higher neutralizing antibody titers against the Omicron variant with booster vaccination doses. Patients who received booster vaccination doses were more frequently asymptomatic

and trended towards less systemic inflammation. While there was no significant statistical difference between vaccination booster status and development of pneumonia found on multivariate analysis, this could be due to the small number of patients in this study.

While several studies are being conducted to continually assess the clinical severity, it is also critical to understand the immunopathological characteristics of infection caused by Omicron. We evaluated in-depth systemic immune response following Omicron breakthrough infections. Vaccine breakthrough infections with the Omicron variant had a low concentration of proinflammatory cytokines, chemokines, and growth factors at the acute phase of infection, in contrast to the high levels reported in severe COVID-19 disease.¹⁴ Instead, a more robust IFN- γ response, which is critical for viral clearance³¹, was detected in these acute Omicron breakthrough infections. These findings corroborated our clinical observations that patients infected with the Omicron variant are mostly asymptomatic or with mild diseases and with a lower viral load. In addition, this emphasizes the importance of vaccination in preventing severe COVID-19 disease with Omicron.

Immunophenotyping of peripheral blood from Omicron breakthrough cases revealed relatively minimal changes in the myeloid cell compartment. Monocytopenia and dysregulated myelopoiesis are typical hallmarks of severe disease and acute respiratory distress syndrome in COVID-19.³²⁻³⁶ We did not detect significant differences in the number of total monocytes and their subsets between patients with Omicron breakthrough and vaccinated healthy individuals, indicating a minimal migration of the monocyte populations into the lung or other sites of inflammation.³⁷ The lower frequency of immature neutrophils in the circulation of these patients, which are released following emergency myelopoiesis in response to ongoing inflammation^{38,39}, further indicates that Omicron breakthrough infections cause a milder inflammatory response.

In addition to circulating antibodies, memory B cells may also provide an additional line of defense against infection.⁴⁰ We previously observed that memory B cell levels were decreased in Delta vaccine breakthrough patients, suggesting that lower levels of memory B cells may have contributed to a failure to protect against infection.⁴¹ In contrast, we observed here that Omicron vaccine breakthrough infections showed a similar frequency of RBD-specific memory B cells as vaccinated healthy individuals. The reasons for this difference remain unclear. However, it is notable that Omicron infection has been suggested to have a shorter incubation period than Delta infection^{42,43} – the faster viral infection and replication speed may mean that memory B cells do not have sufficient time to reactivate into antibody-secreting cells to contribute toward preventing infection.

Given the rapid spread of Omicron across the world, there were concerns of increased infectivity compared with Delta. Chen et al. demonstrated significantly increased binding free affinity of Omicron, suggesting about 2.8 times infectivity compared with Delta.⁴⁴ we showed that initial and lowest median PCR Ct values were higher in Omicron than Delta vaccine breakthrough infections. Our study showed that the lowest Ct values (peak viral load) occurred at similar times for Omicron and Delta vaccine breakthrough infections – after onset of symptoms. These suggest that the apparent increased

transmissibility and infectivity of Omicron infections might not be related to asymptomatic infection or higher viral loads.

There are several limitations to our study. Firstly, due to the high vaccine uptake in Singapore and requirements for vaccination to travel, we were not able to study Omicron infection in unvaccinated individuals. Secondly, while RT-PCR screening of travelers and quarantined individuals was not dependent on symptomatic illness, abortive infections may have been missed. Local transmission of Omicron may have occurred undetected in Singapore over the course of this study; however, this is likely to have been limited in early December 2021 as we did not find S gene target failure in looking back at returned travelers with COVID-19 prior to Omicron being reported in South Africa. Thirdly, initial PCR testing was not standardized in a centralized laboratory. It was performed using various commercially available assays at each center. Ct values are only a surrogate measure of viral load and shedding and might be influenced by several factors. We attempted to evaluate the viability of shed virus via viral culture, however, sample size was small. Finally, this is an observational study and comparison between Delta and Omicron infected cohorts is subjective to additional confounding (such as time from most recent vaccine administered which may affect level of immune waning) that may have affected outcomes.

Conclusion

To date, the majority of infections with Omicron variant in Singapore have been mild, with no associated deaths reported. The rate of viral load decline appears similar to Delta infections in vaccinated individuals and viable virus was isolated during the first five days of illness. This has implications on public health policy. Individuals who received booster vaccination doses were more frequently asymptomatic and had milder systemic inflammation. Widespread vaccination with booster doses retains an essential role in the control of the pandemic and protection against future variants.

Declarations

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Tables

Table 1. Characteristics of age-matched B.1.1.529 and B.1.617.2 cases

Variant: World Health Organization label	B.1.1.529 (Omicron)	B.1.617.2 (Delta)	P-value
N	87	87	
Demographics			
Age, median (IQR), years	34 (29-43)	43 (33-52)	<0.001
Male sex, n (%)	51 (58.6)	54 (62.1)	0.574
Charlson comorbidity index, median (IQR)	0 (0 - 0)	0 (0 - 0)	0.090
Re-infection, n (%)	1 (1.1)	0 (0)	1.000
Median PCR Ct value at diagnosis (IQR)	20.7 (17.9 - 28.5)	19.1 (15.4 - 21.1)	<0.001
Vaccination State			
2 doses, n (%)	71 (81.6)	87 (100.0)	<0.001
>2 doses, n (%)	16 (18.4)	0 (0)	<0.001
mRNA vaccine, n (%)†	78 (89.7)	83 (95.4)	0.061
Symptoms during illness, n (%)			
Asymptomatic	20 (23.0)	27 (31.0)	0.261
Fever	24 (27.6)	36 (41.4)	0.076
Cough	39 (44.8)	32 (36.8)	0.381
Nasal congestion and/or rhinorrhea	30 (34.5)	28 (32.2)	0.588
Sore throat	40 (46.0)	20 (23.0)	0.005
Anosmia	3 (3.4)	2 (2.3)	0.549
Diarrhea	5 (5.7)	0 (0)	0.049
Worst laboratory values, median (IQR) ‡			
Neutrophil, 10 ⁹ /L	2.95 (2.16 - 3.96)	4.60 (3.76 - 6.10)	<0.001
Lymphocyte, 10 ⁹ /L	1.46 (1.02 - 1.80)	1.40 (0.97 - 1.89)	0.678
Alanine aminotransferase, U/L	18 (14 - 31)	18 (13 - 31)	0.938
C-reactive protein, mg/L	5.7 (2.0 - 10.0)	12.0 (6.1 - 22.0)	<0.001
Lactate dehydrogenase, U/L	339 (292 - 377)	356 (313 - 419)	0.02
Polymerase chain reaction cycle threshold	17.6 (16.3 -)	14.9 (13.9 -)	0.001

value	19.3)	19.0)	
Outcome, n (%)			
Pneumonia	3 (3%)	14 (17%)	0.005
Supplemental oxygen	0	2	0.243
Intensive care unit admission and/or death	0	0	-

† Non-mRNA vaccines received included ChAdOx1_nCoV-19 (COVISHIELD), COVAXIN, ChAdOx1-S (Oxford/AstraZeneca) and Sinopharm BBIP-CorV vaccines; ‡ Worst laboratory value defined as the highest recorded admission, except for lymphocyte count in which worst laboratory value is the lowest

Table 2. Characteristics and Disease Outcomes between B.1.1.529 Infected Patients who Received Primary versus Boosted Vaccination

	2 vaccination doses (n=85)	> 2 vaccination doses (n=16)	p-value
Median age, years (IQR)	32 (26 – 39)	46 (31 – 50)	<0.001
Asymptomatic, n (%)	17 (20.0)	5 (31.3)	0.332
Worst laboratory values, median (IQR) †			
Neutrophil, 10 ⁹ /L	3.17 (2.15 – 4.35)	3.03 (2.59 – 3.97)	0.167
Lymphocyte, 10 ⁹ /L	1.45 (1.12 – 1.77)	1.54 (1.23 – 1.93)	0.403
Alanine aminotransferase, U/L	18 (14 – 32)	17 (12 – 36)	0.996
C-reactive protein, mg/L	5.3 (2.2 – 9.8)	2.9 (0.7 – 9.7)	0.152
Lactate dehydrogenase, U/L	345 (303 – 384)	330 (286 – 354)	0.377
Polymerase chain reaction cycle threshold values, median (IQR)			
At illness onset	20.9 (18.2 – 28.4)	23.0 (19.9 – 25.3)	0.934
Lowest	18.2 (16.3 – 20.2)	17.6 (16.5 – 18.5)	0.209
Serological Features			
Median anti-SARS-CoV-2 anti-Spike protein (anti-S) antibodies, U/mL (IQR)	1,570.5 (969.3 – 2141.3)	12,500 (6,435 – 12,500)	<0.001

†Worst laboratory value defined as the highest recorded admission, except for lymphocyte count in which worst laboratory value is the lowest

Figures

Figure 1. Scatterplot of Ct values and marginal effect of day of illness of COVID-19 B.1.1.529 infected patients from generalized additive mixed model compared with B.1.617.2 infected.

Figure 1

See image above for figure legend.

Figure 2. Systemic cytokine responses in Omicron breakthrough cases. Concentrations of 45 immune mediators were quantified using a 45-plex microbead-based immunoassay. **(A)** Principal component analysis (PCA) of 45 immune mediator levels analyzed in acute samples of Omicron vaccine breakthrough cases ($n=8$) compared to their age-matched Delta vaccine breakthrough cases ($n=16$) and healthy vaccinated controls ($n=16$). PC1 explains 21.7% of the variation, while PC2 explains 12.8% of the variation; color denotes different groups of subjects. **(B)** The levels of immune mediators that associated with disease severity in COVID-19 patients were shown as scatter plots. Patient samples with concentration out of measurement range are presented as the value of logarithm transformation of Limit of Quantification. One-way ANOVA test with post-hoc Dunn's multiple comparisons test was performed to compare the immune mediator profiles among the three groups. (* $p < 0.05$; ** $p < 0.01$).

Figure 2

See image above for figure legend.

Figure 3. Whole blood immunophenotyping of Omicron breakthrough cases. (A) Unbiased analysis by Uniform Manifold Approximation and Projection (UMAP) clustering of circulating CD45+ immune cells in vaccinated healthy controls (n=13), Delta vaccine breakthrough acute infections (n=15) and Omicron vaccine breakthrough acute infection (n=17). **(B)** Absolute counts of circulating monocytes, neutrophils and T-cell compartments were presented as scatter plots. Kruskal-Wallis test with post-hoc Dunn's multiple comparisons test was performed to compare the cellular profiles among the three groups. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure 3

See image above for figure legend.

Figure 4. Immunological status of Omicron breakthrough cases. (A) Unbiased analysis by Uniform Manifold Approximation and Projection (UMAP) clustering of circulating CD45+ immune cells in vaccinated healthy controls (n=13), Delta vaccine breakthrough acute infections (n=15) and Omicron vaccine breakthrough acute infection (n=14). (B) Frequency of circulating monocytes, neutrophils and T-cells were presented as scatter plots. Kruskal-Wallis test with post-hoc Dunn's multiple comparisons test was performed to compare the cellular profiles among the three groups. (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure 4

See image above for figure legend.

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