Journal of Neurology (2023) 270:2817–2825
https://doi.org/10.1007/s00415-023-11692-4

ORIGINAL COMMUNICATION

COVID-19 infection after SARS-CoV-2 mRNA vaccination in Multiple Sclerosis, AQP4-antibody NMOSD and MOGAD patients during the Omicron subvariant BA.1/2 wave in Singapore

Tianrong Yeo[1,2,3] · Rachel Wan En Siew[1] · Muhammad Yaaseen Gulam[1] · Janis Siew Noi Tye[1] · Amelia Yun Yi Aw[1] · Thanushiree Sivalingam[1] · Xuejuan Peng[1] · Kok Pin Yong[1] · Seyed Ehsan Saffari[1,2] · Yinxia Chao[1,2,4] · Kevin Tan[1,2]

Received: 21 December 2022 / Revised: 26 March 2023 / Accepted: 27 March 2023 / Published online: 7 April 2023

Abstract

Background The SARS-CoV-2 Omicron variant appears to cause milder infections, however, its capacity for immune evasion and high transmissibility despite vaccination remains a concern, particularly in immunosuppressed patients. Herein, we investigate the incidence and risk factors for COVID-19 infection in vaccinated adult patients with Multiple Sclerosis (MS), Aquaporin-4-antibody Neuromyelitis Optica Spectrum Disorder (AQP4-Ab NMOSD), and Myelin Oligodendrocyte Glycoprotein-antibody associated disease (MOGAD) during the Omicron subvariant BA.1/2 wave in Singapore.

Methods This was a prospective observational study conducted at the National Neuroscience Institute, Singapore. Only patients who had at least two doses of mRNA vaccines were included. Data on demographics, disease characteristics, COVID-19 infections and vaccinations, and immunotherapies were collected. SARS-CoV-2 neutralising antibodies were measured at various time points after vaccination.

Results Two hundred and one patients were included; 47 had COVID-19 infection during the study period. Multivariable logistic regression revealed that receipt of a third SARS-CoV-2 mRNA vaccination (V3) was protective against COVID-19 infection. No particular immunotherapy group increased the risk of infection, however, Cox proportional-hazards regression showed that patients on anti-CD20s and sphingosine-1-phosphate modulators (S1PRMs) had a shorter time to infection after V3, compared to those on other immunotherapies or not on immunotherapy.

Conclusions The Omicron subvariant BA.1/2 is highly infectious in patients with central nervous system inflammatory diseases; three doses of mRNA vaccination improved protection. However, treatment with anti-CD20s and S1PRMs predisposed patients to earlier infection. Future studies are required to determine the protective efficacy of newer bivalent vaccines that target the Omicron (sub)variant, especially in immunocompromised patients.

Keywords COVID · Infection · Vaccination · Multiple Sclerosis · AQP4 · MOGAD

Introduction

The introduction of effective vaccines against the SARS-CoV-2 virus has seen a significant decline in COVID-19 infection-related mortality and morbidity globally [1]. However, the emergence of new SARS-CoV-2 variants (and subvariants) has called into question the continued efficacy of these vaccines in preventing infections, in particular serious infections. The Omicron variant of SARS-CoV-2 was first reported in South Africa in November 2021 and rapidly became the dominant variant circulating globally at the end of 2021 [2]. While the Omicron variant appears to cause milder infections [3], its capacity for immune evasion and

✉ Tianrong Yeo
yeo.tianrong@singhealth.com.sg

1 Department of Neurology, National Neuroscience Institute, Singapore, Singapore

2 Duke-NUS Medical School, Singapore, Singapore

3 Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore

4 Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore

high transmissibility, even within vaccinated individuals, remains a concern [4, 5].

Previous studies have reported that Multiple Sclerosis (MS) patients on anti-CD20 therapies (anti-CD20s) and sphingosine-1-phosphate receptor modulators (S1PRMs) have a higher risk of COVID-19 infection [6–8], and it is now established that patients who are older, male and have higher disability are at an increased risk of severe infection [9]. However, most of these studies were performed during the earlier phases of the COVID-19 pandemic; it is not clear whether these risk factors persist within vaccinated individuals in an environment where new SARS-CoV-2 variants (and subvariants) have emerged. In this study, we aim to investigate the incidence of symptomatic COVID-19 infection in vaccinated adult patients with MS, Aquaporin-4-antibody Neuromyelitis Optica Spectrum Disorder (AQP4-Ab NMOSD) and Myelin Oligodendrocyte Glycoprotein-antibody associated disease (MOGAD) during the Omicron subvariant BA.1/2 wave in Singapore, and to delineate the factors associated with infection.

Methods

Study and follow up periods

This was a prospective observational study conducted at the Department of Neurology, National Neuroscience Institute, Singapore, under local ethics approval (CIRB number 2020/2410, 2021/2222). A study period from 1st January 2022 to 30th April 2022 was specified to define the Omicron subvariant BA.1/2 wave in Singapore. The start date was chosen as this represented the inflection point at which the Delta variant wave ended, followed closely by the surge in Omicron subvariant BA.1/2 infections in Singapore [10, 11]. The study end date was selected as this corresponded to the decline in the incidence of Omicron subvariant BA.1/2 infections before the emergence of the Omicron subvariant BA.4/5 wave; the first community case of the Omicron subvariant BA.4/5 being detected on 15th May 2022. Therefore, it was reasonable to assume the infections that occurred within the study period was predominantly of the Omicron BA.1/2 subvariant.As it was no longer compulsory to see a medical practitioner for the confirmation of COVID-19 infection since January 2022 and the real-time medical records of some patients who had COVID-19 infection did not reflect this, infection status was difficult to establish during the study period. To ensure accurate identification of infected and non-infected individuals, a follow-up period from 1st May to 31st August 2022 was specified, during which patients were prospectively reviewed by the clinical team and ascertained if they had COVID-19 infection during the study period. Patients who contacted the clinical team (via phone or email) from 1st January to 31st August 2022 to inform that they had COVID-19 infection during the study period were also included.

Participants

Only patients who had at least 2 doses of either the BNT162b2 (Pfizer-BioNTech) or mRNA-1273 (Moderna) vaccines were included in this study. Infection was defined as the presence of symptoms consistent with COVID-19 infection with a positive polymerase chain reaction or antigen rapid test. Patients who had prior COVID-19 infection (after at least 2 vaccinations) preceding the current study period were excluded; they ($n = 19$) had been reported in our earlier study on the Delta infection wave (from 1st September to 31st December 2021) [12]. The rationale for their exclusion was that recent infection would have conferred protective immunity that is likely to reduce their infective risk during the current study period and this would also have precluded them from receiving follow up vaccinations in the short term (i.e. during the current study period) as it was recommended to postpone vaccination for at least 3 months after infection according to local guidelines. Additionally, in the event that these patients did receive follow up vaccinations, measurements of their post vaccination neutralising antibodies (NAbs) levels would likely have been altered by recent prior infection (see below).

All MS patients fulfilled the 2017 McDonald criteria [13], and all AQP4-NMOSD and MOGAD patients had positive antibodies tested using cell-based assays. Data on demographics, disease characteristics, SARS-CoV-2 mRNA vaccinations, COVID-19 infection, and immunotherapies were collected. Immunotherapies were classified into 5 groups: (1) anti-CD20s and S1PRMs; (2) disease-modifying anti-rheumatic drugs (DMARDs); (3) immune reconstitution therapies (IRTs); (4) other disease-modifying therapies (DMTs); and (5) nil (refer to Supplemental Material for the full list of medications under each immunotherapy group). Anti-CD20s and S1PRMs were grouped together as these treatments are associated with attenuated humoral responses post SARS-CoV-2 vaccination; additionally, emerging studies have demonstrated an increased rate of breakthrough COVID-19 infections in patients on these therapies despite vaccinations [6–8]. We established strict criteria to determine if a patient was ascribed to a particular immunotherapy; the immunotherapy must be commenced at least 3 months prior to first vaccination (V1) and maintained till the administration of V2. Patients who had received IRTs were ascribed to these treatments regardless of the duration between treatment and V1, while individuals on anti-CD20s were classified under these treatments if they had received treatment within 8 months prior to V1. Untreated patients were ascribed as such if they had not been on any

immunotherapy for at least 3 months prior to V1 and maintained till V2.

Measurement of SARS-CoV-2 neutralising antibodies

We determined the levels of NAbs against SARS-CoV-2 in patients at the following time points during their routine clinical reviews: (1) at 2–6 weeks post V2; (2) at 8–16 weeks post V2; and at (3) 2–16 weeks post V3. NAbs were measured using the Genscript® cPass™ surrogate virus neutralisation test according to the manufacturer's instructions. NAbs levels were expressed quantitatively as inhibition percentage; greater values indicated higher levels of NAbs. An inhibition percentage of ≥ 30% represented a detectable NAbs response, as per the manufacturer's technical specifications.

Statistical analysis

Statistical analysis and graphical representation were performed using SAS software version 9.4 for Windows (Cary, NC: SAS Institute Inc) and GraphPad Prism (version 6, GraphPad Software, San Diego, California USA). Univariate and multivariable logistic regression were performed to identify potential factors associated with an increased risk of COVID-19 infection, and odds ratios (OR) along with 95% confidence intervals (CI) were calculated. The goodness-of-fit of the logistic regression models were assessed via Hosmer and Lemeshow test. Cox proportional-hazards regression was conducted to investigate the association of variables of interest with time to COVID-19 infection, and the results were expressed as hazard ratios (HR) with 95% CI. The adequacy of the Cox regression model was assessed via checking the proportional hazards assumption by cumulative sums of martingale residuals over follow-up times or covariate values. For both logistic and Cox regression models, Firth's penalised likelihood approach was applied to reduce bias in parameter estimates. NAbs levels between immunotherapy groups were compared using one-way analysis of variance with post-hoc Bonferroni correction. Statistical significance was set at $p < 0.05$.

Results

Study population

Two hundred and one patients who completed at least 2 doses of SARS-CoV-2 mRNA vaccination met the inclusion criteria—125 (62.2%) MS, 65 (32.2%) AQP4-NMOSD and 11 (5.5%) MOGAD, the proportions of which were consistent with recent local disease epidemiological data [14]. Fifty-four (26.9%) patients were on anti-CD20s and S1PRMs, 51 (25.4%) on DMARDs, 20 (10.0%) on IRTs, 22 (10.9%) on other DMTs, and 54 (26.9%) were not on immunotherapy. Prior to the study end date, 176 patients (87.6%) had received V3 (Pfizer BioNTech 144/176 [81.8%]; Moderna 32/176 [18.2%]) at a median of 24.3 weeks (interquartile range [IQR] 20.9–27.5) from V2, while 8 individuals had taken V4. Figure 1 illustrates the bar chart of the number of patients who received V3 by month within the study cohort.

COVID-19 infections

Forty-seven COVID-19 infections occurred during the study period. Figure 1 shows the number of infections stratified by month—4 infections occurred in January 2022, 21 in February, 17 in March, and 5 in April. This mirrored the infection rates in the Singaporean population during the Omicron subvariant BA.1/2 wave that began in early January 2022, peaking towards the end of February with a 7-day rolling average of over 18,000 new cases, before declining and reaching a nadir in end April [10, 11]. Forty-five (95.7%) infected patients had mild infections while 2 (4.3%) had infections of moderate severity—both patients were on Rituximab at V1 receipt and at the time of infection; 1 of whom was hospitalised. No patients required oxygen supplementation or high-dependency care and all patients

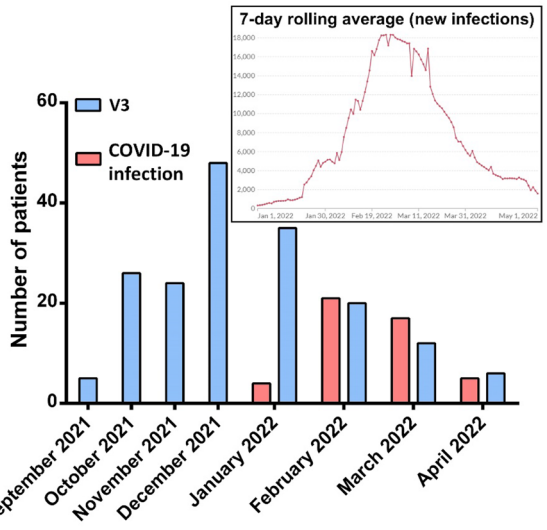

Fig. 1 SARS-CoV-2 mRNA vaccinations and COVID-19 infections in the study cohort by month. Blue: number of V3 received within the cohort from September 2021 (the earliest receipt of V3 was on 20th September 2021) till April 2022. Red: number of COVID-19 infections during the study period (i.e. 1st January to 30th April 2022). Boxed inset: 7-day rolling average of new infections in Singapore during the study period (data obtained from references 10 and 11 with permissions). *V3* third mRNA vaccination

recovered. Three patients received antiviral medication (i.e. Paxlovid); 2 with mild infections and 1 with infection of moderate severity. Fourteen (29.8%) of the infected patients were on anti-CD20s—10 were on Rituximab and 4 on Ocrelizumab, with a median duration from last infusion to infection of 17.3 weeks (IQR 11.1–24.4). Four (8.5%) infected patients were on S1PRMs (all on Fingolimod). In total, one third (18/54) of all patients on anti-CD20s and S1PRMs were infected. The clinical details of infected and uninfected patients are tabulated in Table 1.

Protective and risk factors for COVID-19 infection

To identify factors associated with an increased risk of COVID-19 infection, we performed univariate logistic regression using COVID-19 infection as a binary outcome with the clinical parameters as independent variables. This revealed that the non-receipt of V3 was significantly associated with COVID-19 infection (OR 5.401, 95% CI 2.252–12.95, $p = 0.0002$), while a trend towards significance was observed for immunotherapy group (omnibus $p = 0.099$), female gender (OR 3.092, 95% CI 0.781–12.24,

Table 1 Demographic and clinical characteristics of the study population, with univariate logistic regression of these characteristics (independent variables) for COVID-19 infection (outcome)

Variable	Infected ($n = 47$)	Uninfected ($n = 154$)	Univariate logistic regression (outcome: infection)			
			Event vs. reference level	Odds ratio (95% CI)	*p* value	Omnibus *p* value
Age, years (median [IQR])	39.2 (32.3– 46.1)	44.0 (32.8– 55.4)	–	0.981 (0.984–1.017)	0.118	
Female, no. (%)	45 (95.7)	132 (85.7)	Female vs. male	3.092 (0.781–12.24)	0.108	
Diagnosis	–	–	–	–	–	0.570
MS, no. (%)	32 (68.1)	93 (60.4)	–	–	–	
AQP4-Ab NMOSD, no. (%)	14 (29.8)	51 (33.1)	AQP4-NMOSD vs. MS	0.810 (0.398– 1.649)	0.561	
MOGAD, no. (%)	1 (2.1)	10 (6.5)	MOGAD vs. MS	0.411 (0.066– 2.566)	0.341	
EDSS ≥ 6, no. (%)	7 (14.9)	28 (18.2)	EDSS ≥ 6 vs. EDSS < 6	0.809 (0.332–1.970)	0.640	
Immunotherapy group	–	–	–	–	–	0.099
Anti-CD20s, S1PRMs, no. (%)	18 (38.3)	36 (23.4)	Anti-CD20s, S1PRMs vs. Nil	1.917 (0.807– 4.555)	0.140	
DMARDs, no. (%)	6 (12.8)	45 (29.2)	DMARDs vs. Nil	0.540 (0.188– 1.556)	0.254	
IRTs, no. (%)	7 (14.9)	13 (8.4)	IRTs vs. Nil	2.102 (0.682– 6.477)	0.196	
Other DMTs, no. (%)	5 (10.6)	17 (11.0)	Other DMTs vs. Nil	1.189 (0.366– 3.865)	0.774	
Nil, no. (%)	11(23.4)	43 (27.9)	–	–	–	
Received Pfizer-BioNTech vaccine for V1 and V2, no. (%)	41 (87.2)	135 (87.7)	Pfizer-BioNTech vs. Moderna	0.919 (0.348– 2.425)	0.865	
Not received V3, no. (%)	14 (29.8)	11 (7.1)	Not received vs. received	5.401 (2.252–12.95)	**0.0002**	
Neutralising antibodies	–	–	–	–	–	
2–6 weeks post V2, inhibition % (median [IQR])*	18.2 (0–94.3)	67.5 (20.9–96.1)	–	0.988 (0.974– 1.003)	0.109	
8–16 weeks post V2, inhibition % (median [IQR])**	85.1 (10.3–96)	82.3 (22.1–94.1)	–	1.000 (0.984– 1.017)	0.991	
Detectable NAbs response within 2–16 weeks post V2 (i.e. ≥ 30% inhibition), no. (%)***	16/27 (59.3)	50/72 (69.4)	Detectable NAbs response vs. negative response	0.639 (0.257– 1.593)	0.337	

*Data available for 58 patients: 19 infected and 39 uninfected. **Data available for 53 patients: 12 infected and 41 uninfected. ***Data available for 99 patients: 27 infected and 72 uninfected. The number of patients in the 'Detectable NAbs response within 2–16 weeks post V2' analysis was lesser than the number of patients in both the '2–6 weeks post V2' and '8–16 weeks post V2 inhibition %' analyses combined because some patients had NAbs measured at both the '2–6 weeks' and '8–16 weeks' time points but they contributed to only one data point for the 'Detectable NAbs response within 2–16 weeks post V2' analysis

Anti-CD20s anti-CD20 therapies, *AQP4-Ab NMOSD* aquaporin-4-antibody Neuromyelitis Optica Spectrum Disorder, *DMARDs* disease-modifying anti-rheumatic drugs, *DMTs* disease-modifying therapies, *EDSS* expanded disability status scale, *IQR* inter-quartile range, *IRTs* immune reconstitution therapies, *MOGAD* myelin oligodendrocyte glycoprotein-antibody associated disease, *MS* multiple sclerosis, *NAbs* neutralising antibodies, *S1PRMs* sphingosine-1-phosphate receptor modulators, *V1* first mRNA vaccination, *V2* second mRNA vaccination, *V3* third mRNA vaccination

$p = 0.108$), and NAbs levels 2–6 weeks post V2 (median [IQR]; infected 18.2% [0.0–94.3] versus uninfected 67.5% (20.9–96.1); OR 0.988, 95% CI 0.974–1.003, $p = 0.109$) (Table 1). When stratified by immunotherapy groups, NAbs levels at 2–6 weeks post V2 were lower in individuals on anti-CD20s and S1PRMs compared with patients on other immunotherapies and untreated patients (Fig. 2a); a similar observation was made at 8 to 16 weeks after V2 (Fig. 2b).

To further delineate the effect of non-receipt of V3 on COVID-19 infection, we proceeded with multivariable logistic regression, adjusting for gender and immunotherapy group, and confirmed that not receiving V3 was a significant independent risk factor for infection (adjusted OR 4.377, 95% CI 1.742–11.00, $p = 0.002$) (Table 2). We did not include NAbs levels (at 2–6 weeks post V2) in this modelling as only over a quarter of patients had NAbs measurements performed, in addition, multicollinearity was likely to be present between NAbs levels and immunotherapy group.

While V3 appeared to be a protective against infection, it was possible that those who received V3 managed to do so because they did not get COVID-19 infection during the study period (i.e. confounding by indication bias). However, this was unlikely as 58.5% (103/176) of the study cohort had received V3 before the start of the study period. To further address this potential bias, we calculated the crude cumulative risk of patients who had received only V2 ($n = 25$) versus those who had V3 ($n = 176$). For those who had only V2, the time-at-risk for infection was from V2 to infection

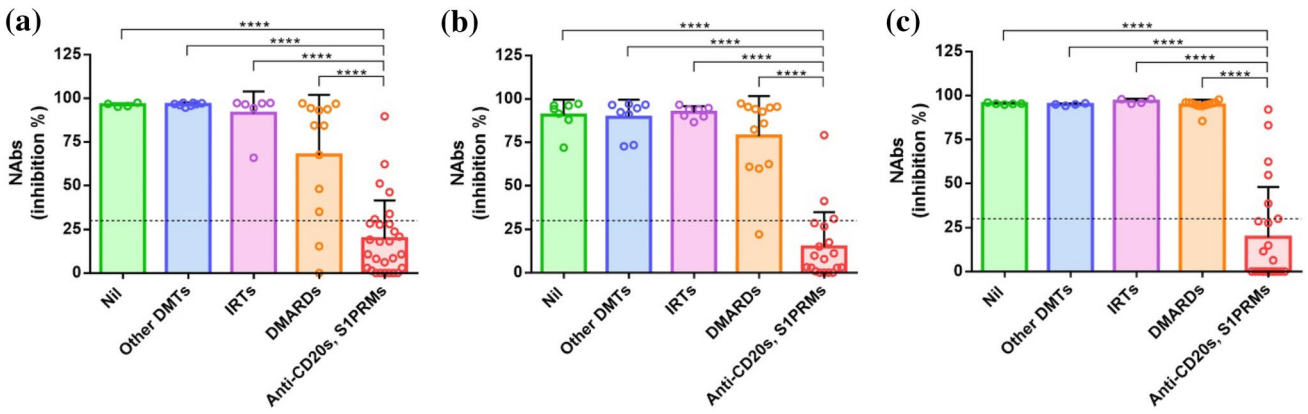

Fig. 2 Mean NAbs levels after V2 and V3, stratified by immunotherapy groups. Lower NAbs levels were observed in patients on anti-CD20s and S1PRMs at (**a**) 2–6 weeks post V2, (**b**) 8–16 weeks post V2, and (**c**) 2–16 weeks post V3, compared with patients on other immunotherapies and without immunotherapy. Dashed line indicates 30% inhibition; values at or above this indicate a detectable NAbs response as specified by the assay manufacturer. ****$p < 0.0001$ by Bonferroni post hoc test after one-way analysis of variance. Error bars represent standard deviation. *Anti-CD20s* anti-CD20 therapies, *DMARDs* disease-modifying anti-rheumatic drugs, *DMTs* disease-modifying therapies, *IRTs* immune reconstitution therapies, *NAbs* neutralising antibodies, *S1PRMs* sphingosine-1-phosphate receptor modulators, *V2* second mRNA vaccination, *V3* third mRNA vaccination

Table 2 Multivariable logistic regression modelling for COVID-19 infection

Variable	Multivariable logistic regression (outcome: infection)			
	Event vs. reference level	Odds ratio (95% CI)	*p* value	Omnibus p value
Female	Female vs. male	2.549 (0.641–10.13)	0.184	
Immunotherapy group	–	–	–	0.253
Anti-CD20s, S1PRMs	Anti-CD20s, S1PRMs vs. Nil	1.840 (0.748–4.527)	0.193	
DMARDs	DMARDs vs. Nil	0.611 (0.207–1.805)	0.065	
IRTs	IRTs vs. Nil	1.688 (0.505–5.639)	0.489	
Other DMTs	Other DMTs vs. Nil	1.561 (0.466–5.226)	0.604	
Non-receipt of V3	Not received vs. received	4.377 (1.742–11.00)	**0.002**	

Overall model *p* value = 0.0007. Model performance was assessed via Hosmer and Lemeshow goodness-of-fit test (*p* value = 0.949)

Anti-CD20s anti-CD20 therapies, *DMARDs* disease-modifying anti-rheumatic drugs, *DMTs* disease-modifying therapies, *IRTs* immune reconstitution therapies, *S1PRMs* sphingosine-1-phosphate receptor modulators, *V3* third mRNA vaccination

for infected patients, while this was from V2 to 30th April 2022 (i.e. censored at the study end date) for uninfected individuals. In those who received V3, the time-at-risk for infection was from V3 to infection for infected patients, and for uninfected patients, this was from V3 to 30th April 2022 or to V4 (if V4 was given within the study period), whichever came first. This analysis revealed that in patients who had only V2, there were 14 infections in 642.1 person-weeks (i.e. 21.8 [95% CI 10.4–33.2] infections/1000 person-weeks). In contrast, in those who had received V3, 33 infections occurred in 2895.9 person-weeks, translating to 11.4 (95% CI 7.5–15.3) infections/1000 person-weeks. If the crude cumulative risk after V3 was assumed to be similar to that after V2, an estimated 63.1 infections (95% CI 30.1–96.2) infections would have occurred during the time-at-risk post V3, however only 33 infections occurred. Therefore, the protective effect of V3 as elucidated from our regression models was unlikely to be confounded by indication bias.

Time to COVID-19 infection

Although there seemed to be an overall effect of immunotherapy group on COVID-19 infection (Table 1), our analysis did not demonstrate any particular immunotherapy group (referenced to no immunotherapy) to have an increased risk of COVID-19 infection during the Omicron subvariant BA.1/2 wave. However, it was possible that certain immunotherapies may predispose patients to earlier infection. To investigate this, we performed Cox proportional-hazards regression using COVID-19 infection as the event and the various clinical parameters as independent variables. We restricted our analysis to only patients who had received V3 (i.e. a standardised start point) and had no change in immunotherapy from V1 through to V3 to ensure consistency in immunotherapy exposure at all vaccination time points. A total of 157 patients were included in this analysis; 30 were infected and 127 uninfected. Time-at-risk of infection was defined as such: for infected patients, this was from V3 to infection; for uninfected patients, this was from V3 to 30th April 2022 or to V4 (if V4 was given within the study period), whichever came first. Univariate Cox regression revealed that patients on anti-CD20s and S1PRMs had a faster time to infection after V3 (HR 3.201, 95% CI 1.150–8.907, $p = 0.026$) (Table 3); this remained significant after adjusting for age (adjusted HR 3.200, 95% CI 1.189–9.269, $p = 0.022$). In 48 patients who had NAbs levels measured at 2–16 weeks after V3, a detectable NAbs response (i.e. ≥ 30% inhibition) appeared to be associated with a longer time to infection (HR 0.464, 95% CI 0.182–1.183, $p = 0.108$), however, the underlying proportional hazard assumption was not satisfied due to the small sample size. Similar to observations made after V2, NAbs levels at 2–16 weeks post V3 were lower in individuals on

Table 3 Univariate Cox regression for time to infection post V3 in 157 patients (30 infected, 127 uninfected) who had no change in immunotherapy group from V1 through to V3

Variable	Univariate Cox regression (outcome: time to infection post V3)			
	Event vs. reference level	Hazard ratio (95% CI)	*p* value	Omnibus *p* value
Age	Year	0.978 (0.952–1.004)	0.103	
Female	Female vs. male	2.198 (0.589–8.198)	0.241	
Diagnosis	–	–	–	0.642
	AQP4-Ab NMOSD vs. MS	0.745 (0.330–1.682)	0.479	
	MOGAD vs. MS	0.541 (0.099–2.949)	0.477	
EDSS	EDSS ≥ 6 vs. EDSS < 6	1.025 (0.422–2.490)	0.956	
Immunotherapy group	–	–	–	0.059
	Anti-CD20s, S1PRMs vs. Nil	3.201 (1.150–8.907)	**0.026**	
	DMARDs vs. Nil	0.853 (0.249–2.920)	0.800	
	IRTs vs. Nil	1.419 (0.299–6.738)	0.660	
	Other DMTs vs. Nil	1.956 (0.530–7.226)	0.314	
Detectable NAbs response within 2 – 16 weeks post V3 (i.e. ≥ 30% inhibition)*	Detectable NAbs response vs. negative response	0.464 (0.182– 1.183)	0.108	

*Data available for 48 patients: 5 infected and 43 uninfected. For the 5 infected patients, there was no correlation between NAbs levels and time to infection ($r = -0.226$, $p = 0.715$)

The adequacy of the Cox regression model was assessed via proportional hazard assumption by Kolmogorov-type supremum test of 1000 simulated patterns. Only 'Detectable NAbs response within 2–16 weeks post V3' variable could not satisfy the underlying assumption which was due to the small sample size

anti-CD20s and S1PRMs compared with those on other immunotherapies or not on immunotherapy (Fig. 2c).

As patients on DMARDs, IRTs and other DMTs did not have a shorter to infection (as compared to patients not on immunotherapy), we combined these 3 treatment groups into a single group (termed 'all others') and proceeded with univariate Cox regression to compare time to infection between patients on (1) anti-CD20s and S1PRMs, (2) 'all others', and (3) no immunotherapy. This showed that patients on 'all others' treatments (HR 0.342, 95% CI 0.155–0.758, $p=0.008$) and patients with no immunotherapy (HR 0.314, 95% CI 0.114–0.859, $p=0.024$) had longer time to infection, compared with individuals on anti-CD20s and S1PRMs (Fig. 3). When referenced to patients without immunotherapy, the HR for patients on anti-CD20s and S1PRMs was 3.189 (95% CI 1.164–8.738, $p=0.024$); this was 3.324 (95% CI 1.208–9.145, $p=0.020$) after adjusting for age. There was no difference between patients with no immunotherapy versus 'all others' (HR 1.092, 95% CI 0.385–3.098, $p=0.869$).

Discussion

In this study, we evaluated the incidence and factors associated with COVID-19 infection in vaccinated patients with MS, AQP4-Ab NMOSD and MOGAD during the Omicron subvariant BA.1/2 wave in Singapore. Consistent with observations made in the Singaporean populace [10, 11], we noted a corresponding surge in new infections within our patient cohort. Our previous study of vaccinated patients during the Delta variant wave showed a cumulative infection incidence of 5.2% (19 infections in 365 patients) over a 4-month period (from September to December 2021) [12], while this was 23.4% (47 infections in 201 patients) ($p<0.001$, chi-square test) for the Omicron BA.1/2 wave over a similar duration. More infections occurred in spite of the fact that a higher proportion of patients had already received V3 during the Omicron BA.1/2 wave (87.6%, 176/201) compared with during the Delta variant wave (47.1%, 172/365) ($p<0.001$, chi-square test). Our result is similar to the CovaXiMS cohort in Italy (which delineated infections during the Omicron and Delta waves) where the Omicron variant was found to increase the risk of infection by about 6 times above that during the Delta variant wave [7]. While the Omicron variant appeared to be highly transmissible and infective, it is reassuring that most infections in our cohort were mild (45/47 [95.7%]), in keeping with observations from MS patient cohorts worldwide [7, 15]. Indeed, for our patients on anti-CD20s (which have been reported to have an increased risk of severe infections during previous infection waves) [16], 85.7% (12/14) were mild infections.

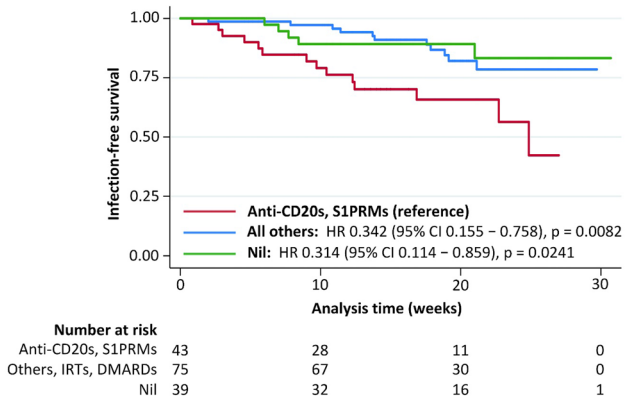

Fig. 3 Kaplan–Meier survival curves showing the time to Omicron BA.1/2 infection after V3 across the 3 immunotherapy groups: (1) anti-CD20s and S1PRMs; (2) 'all others' (DMARDs, IRTs, other DMTs); and (3) no immunotherapy. Patients on anti-CD20s and S1PRMs had a shorter time to infection compared with the other 2 groups. HRs indicated are from univariate Cox regression with the anti-CD20s and S1PRMs group as reference. *Anti-CD20s* anti-CD20 therapies, *DMARDs* disease-modifying anti-rheumatic drugs, *DMTs* disease-modifying therapies, *HR* hazard ratio, *IRTs* immune reconstitution therapies, *S1PRMs* sphingosine-1-phosphate receptor modulators, *V3* third mRNA vaccination

Several of our findings also mirror those made in the Italian CovaXiMS cohort, chiefly amongst them, the demonstration that the receipt of V3 was protective against infection during the Omicron subvariant BA.1/2 wave. This is important from a public health perspective as it provides evidence to support the current strategy of continued vaccinations to prevent infections (and indeed severe infections) in patients with neuroinflammatory diseases, especially those who are immunocompromised, even as new (sub)variants emerge. Secondly, while there is female preponderance in MS and AQP4-Ab NMOSD, we observed that a higher proportion of females were infected during the Omicron BA.1/2 wave compared with the Delta variant wave; 95.7% (45/47) versus 68.4% (13/19, data from our previous study of the Delta wave) ($p=0.006$, Fisher's exact test) [12]. This was likewise observed in the CovaXiMS cohort in which 74.5% (73/98) of those infected during the Omicron wave were female, in contrast to 54.5% (18/33) during the Delta wave [7]. It is not yet fully understood whether sex-based biological factors are determinants of Omicron infection, much less in individuals who are vaccinated and/or are on immunomodulatory medications. Social factors such as the rate of contacts following relaxation of COVID-19 social restrictions and health-seeking behaviour may also contribute to gender differences in infection incidence during the Omicron wave [17, 18]. Lastly, we noted that age, higher disability, and mRNA vaccine brand did not have any effect on the risk of Omicron infection, as reported likewise in the CovaXiMS cohort [7].

We observed that patients on anti-CD20s and S1PRMs had a shorter time to Omicron subvariant BA.1/2 infection even after V3 (compared with patients on other

immunotherapies or not on immunotherapy), although they did not have a higher cumulative infection incidence throughout the entire duration of the Omicron BA.1/2 wave. These results are similar to the CovaXiMS study which showed shorter time to Omicron infection after V2 in anti-CD20s treated patients while demonstrating a decrease in the proportion of infected patients on anti-CD20s (Delta: 48.5%, Omicron 36.7%) and Fingolimod (Delta: 18.2%, Omicron: 8.2%) during the Omicron wave [7]. These lines of evidence imply that patients on other immunotherapies are also acquiring Omicron infections, albeit later. This is likely to be explained by the reduced/waning protective effect of post-vaccination immune responses (developed against previous variants) against Omicron infection [19]. Post-vaccination humoral response can be assessed by measuring the levels of receptor-binding domain (RBD) or neutralising (NAbs) antibodies. The CovaXiMS study showed that while higher RBD antibody levels (measured at 4 weeks post V2) were still associated with reduced infection risk during the Omicron wave, this protective effect was decreased by ~40% [7], suggesting that antibody response may be a less important determinant of infection during the Omicron wave compared with previous waves. We observed lower NAbs levels measured at 2–6 weeks post V2 in infected patients (versus uninfected) although this was not statistically significant, likely due to the small sample size (~30% of the cohort had NAbs measured at this time point). There was no difference in NAbs levels measured at a later time point, i.e. 8–16 weeks post V2, stratified by infection status. To date, no antibody threshold has been identified to confer absolute protection from COVID-19 infection and this is unlikely to be as the pandemic continues, in view of waning humoral response over time (and indeed at the time of infection) and the role of other immune components (e.g. T cell-mediated processes), modified by host biological factors and social considerations as specified above.

Our study's main limitation is the small number of infected cases which limited the power of the study. Second, we were not able to comprehensively sample the whole study cohort to assess their NAbs responses, particularly post V2, which was in part due to restrictions on clinical and research visits during that phase of the COVID-19 pandemic. This precluded the robust inclusion of NAbs levels into our statistical models. Similarly, we did not assess post-vaccination T-cell immune responses which would have added clarity to its protective contribution against Omicron infection. The presence of a control group of unvaccinated patients to compare the effects of the Omicron BA.1/2 wave would have been informative; however, this was unavailable due to the high rate of SARS-CoV-2 vaccination within the Singaporean populace (~91% of the population had received V2 at the start of the study period) and amongst our patients [11]. There are several strengths in our study. We had strict criteria for patients to be ascribed to immunotherapy groups so that we could delineate the effect of treatment on infection and the inclusion of a follow up period allowed robust data capture, particularly with regards to infection status. Other notable strengths include the analysis of AQP4-Ab NMOSD and MOGAD patients which meant that the effect of DMARDs could be evaluated. Finally, the fact that Singapore is a small, densely populated city state reduced the variability in the degree of viral exposure amongst individuals in the study.

The COVID-19 epidemic has now transitioned to an endemic phase, and with this, the emergence of more (sub) variants, e.g. Omicron subvariants BA.4/5, BQ.1, XBB. In Singapore, bivalent vaccines that target several Omicron subvariants are now available as booster vaccinations. Recent reports have demonstrated that these bivalent vaccines conferred additional protection against symptomatic SARS-CoV-2 infection during the circulation of BA.4/BA.5 in the United States, compared with monovalent vaccines alone [19]. Future studies are required to determine if the protective efficacy of these newer vaccines extend to patients on immunosuppressive therapies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00415-023-11692-4.

Acknowledgements We thank all our patients for participating in the study.

Author contributions TY: Conceptualisation; Formal analysis; Investigation; Methodology; Project administration; Resources; Supervision; Visualisation; Writing – original draft. RWES: Data curation; Investigation; Writing—review & editing. MYG: Investigation; Writing – review & editing. JSNT: Investigation; Writing – review & editing. AYYA: Investigation; Writing – review & editing. TS: Investigation; Writing – review & editing. XP: Investigation; Writing – review & editing. KPY: Investigation; Writing – review & editing. SES: Formal analysis; Visualisation; Writing – review & editing. YC: Investigation; Resources; Writing – review & editing. KT: Investigation; Resources; Writing – review & editing. All authors read and approved the final manuscript.

Funding This study was supported by the National Neuroscience Institute (NNI) Neuroimmunology Academic Fund. Tianrong Yeo is funded by the Singapore Ministry of Health's National Medical Research Council Transition Award (MOH-TA20nov-002). Yinxia Chao is funded by the National Medical Research Council (NMRC/CSAINV20nov-0015 and OFLCG18May-0026).

Data availability Anonymised data can be made available to qualified investigators on reasonable request.

Declarations

Conflicts of interest Tianrong Yeo has received honoraria from ASNA, Edanz Pharma, Euroimmun AG, Merck, Novartis, Terumo BCT for consulting services and speaker's fees, and research grants from the National Medical Research Council (NMRC Singapore) and Roche. He has also received travel grants from UCB, Merck and PACTRIMS, and travel awards from ACTRIMS, ECTRIMS and Orebro University.

Kevin Tan has received travel grants and compensation from Novartis, Merck, Sanofi, Eisai, Viela Bio and Roche for consulting services. Rachel Wan En Siew, Muhammad Yaaseen Gulam, Janis Siew Noi Tye, Amelia Yun Yi Aw, Thanushiree Sivalingam, Xuejuan Peng, Kok Pin Yong, Seyed Ehsan Saffari, and Yinxia Chao report no competing interests.

Ethical approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. This study was approved by the Institutional Review Board at SingHealth (CIRB number 2020/2410, 2021/2222).

Informed consent Written informed consent was obtained from all patients in the study.

References

1. Watson OJ et al (2022) Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. Lancet Infect Dis 22(9):1293–1302. https://doi.org/10.1016/S1473-3099(22)00320-6
2. Viana R et al (2022) Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. Nature 603(7902):679–686. https://doi.org/10.1038/s41586-022-04411-y
3. Menni C et al (2022) Symptom prevalence, duration, and risk of hospital admission in individuals infected with SARS-CoV-2 during periods of omicron and delta variant dominance: a prospective observational study from the ZOE COVID Study. Lancet 399(10335):1618–1624. https://doi.org/10.1016/S0140-6736(22)00327-0
4. Jalali N et al (2022) Increased household transmission and immune escape of the SARS-CoV-2 Omicron compared to Delta variants. Nat Commun 13(1):5706. https://doi.org/10.1038/s41467-022-33233-9
5. Hu J et al (2022) Increased immune escape of the new SARS-CoV-2 variant of concern Omicron. Cell Mol Immunol 19(2):293–295. https://doi.org/10.1038/s41423-021-00836-z
6. Rose DR et al (2021) Clinical features and outcomes of COVID-19 despite SARS-CoV-2 vaccination in people with multiple sclerosis. Mult Scler J Exp Transl Clin 7(4):20552173211057110. https://doi.org/10.1177/20552173211057110
7. Sormani MP et al (2022) Breakthrough SARS-CoV-2 infections after COVID-19 mRNA vaccination in MS patients on disease modifying therapies during the Delta and the Omicron waves in Italy. EBioMedicine 80:104042. https://doi.org/10.1016/j.ebiom.2022.104042
8. Schiavetti I et al (2022) Breakthrough SARS-CoV-2 infections in MS patients on disease-modifying therapies. Mult Scler 28(13):2106–2111. https://doi.org/10.1177/13524585221102918
9. Simpson-Yap S et al (2022) Updated results of the COVID-19 in MS global data sharing initiative: anti-CD20 and other risk factors associated with COVID-19 severity. Neurol Neuroimmunol Neuroinflamm. https://doi.org/10.1212/NXI.0000000000200021
10. Dong E, Du H, Gardner L (2020) An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 20(5):533–534. https://doi.org/10.1016/S1473-3099(20)30120-1
11. Mathieu E et al. Coronavirus Pandemic (COVID-19). Published online at OurWorldInData.org. Retrieved from: https://ourworldindata.org/coronavirus
12. Yeo T et al (2022) COVID-19 infection after two doses of SARS-CoV-2 mRNA vaccine in multiple sclerosis AQP4-antibody NMOSD and MOGAD. Mult Scler Relat Disord 65:104003. https://doi.org/10.1016/j.msard.2022.104003
13. Thompson AJ et al (2018) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 17(2):162–173. https://doi.org/10.1016/S1474-4422(17)30470-2
14. Tan K et al (2021) Central nervous system inflammatory demyelinating diseases and neuroimmunology in Singapore—Epidemiology and evolution of an emerging subspecialty. Neurol Clin Neurosci 9(4):259–265. https://doi.org/10.1111/ncn3.12479
15. Paybast S, Habibi MA, Naser Moghadasi A (2022) Characteristics and management of multiple sclerosis patients during the Omicron era: is there a concern about the MS course in the face of the new variant of COVID-19? Neurol Sci. https://doi.org/10.1007/s10072-022-06447-4
16. Schiavetti I et al (2022) Severe outcomes of COVID-19 among patients with multiple sclerosis under anti-CD-20 therapies: a systematic review and meta-analysis. Mult Scler Relat Disord 57:103358. https://doi.org/10.1016/j.msard.2021.103358
17. Doerre A, Doblhammer G (2022) The influence of gender on COVID-19 infections and mortality in Germany: Insights from age- and gender-specific modeling of contact rates, infections, and deaths in the early phase of the pandemic. PLoS One 17(5):e0268119. https://doi.org/10.1371/journal.pone.0268119
18. Galasso V et al (2020) Gender differences in COVID-19 attitudes and behavior: Panel evidence from eight countries. Proc Natl Acad Sci U S A 117(44):27285–27291. https://doi.org/10.1073/pnas.2012520117
19. Link-Gelles R et al (2022) Effectiveness of bivalent mRNA vaccines in preventing symptomatic SARS-CoV-2 infection - increasing community access to testing program, United States, September-November 2022. MMWR Morb Mortal Wkly Rep 71(48):1526–1530. https://doi.org/10.15585/mmwr.mm7148e1