Viral load dynamics in SARS-CoV-2 Omicron breakthrough infections

Running title: **Viral dynamics in Omicron infections**

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Footnote Page

1) The authors declare no conflict of interest
2) This research received no external funding
3) Not Applicable
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Abstract

In order to determine viral dynamics in Omicron breakthrough infections, we measured SARS-CoV-2 RNA in 206 double vaccinated or boostered individuals. During the first three days following the onset of symptoms, viral loads were significantly higher (Ct 21.76) in vaccinated compared to boostered (Ct 23.14) individuals ($p = 0.029$). However, by performing a longitudinal analysis on 32 individuals over 14 days, no difference in the viral load trajectory was observed between double vaccinated and boostered patients. Our results indicate that booster immunization results in a small reduction in detectable viral loads without significantly changing viral load dynamics over time.

Keywords

Omicron, breakthrough infection, viral load, booster immunization, vaccination

Background

The Omicron variant of SARS-CoV-2 is characterized by a significant immune escape resulting in reduced vaccine effectiveness and a surge of breakthrough infections [1–3]. To date, the Omicron VOC has become the dominant SARS-CoV-2 variant worldwide. The dynamics of viral shedding can critically determine the risk of transmitting SARS-CoV-2. Differences in viral load dynamics have been reported for various SARS-CoV-2 variants including Delta that was demonstrated to cause higher peak viral loads compared to SARS-CoV-2 lineage A/B infections [4,5]. Furthermore, vaccination status can impact peak viral loads as well as the duration of viral shedding [6]. Therefore, vaccination is of critical importance to reduce the rate of transmissions and to contain infections. Given the high numbers of Omicron breakthrough infections and the subsequent challenge to maintain critical infrastructure, it is essential to understand the characteristics and dynamics of viral loads and their dependence on vaccination status.

Methods

Ethical considerations

The project outline was submitted to the Institutional Review Board (IRB) of the University of Cologne which stated the investigators were exempt from applying for ethical approval because analysis were solely retrospective.

Cross-sectional cohort

Viral load measurements and information on symptoms were documented as part of the standard diagnostic procedure at the COVID-19 test center of the University Hospital Cologne.

Longitudinal cohort

Viral loads were monitored for quality control. The high number of Omicron breakthrough infections suggested that a significant fraction of the employees of the University Hospital would not be able to work due to isolation. Thus, determining the duration of infectivity was crucial for maintaining the critical infrastructure. Follow-up detection of viral loads was performed after verbal consent.

Study Population

Study participants were outpatients or employees tested at the COVID-19 test center of the University Hospital Cologne. Individuals presented based on their onset of symptoms or due to contact with infected individuals.

Cross-sectional cohort

Participants were included based on the availability of information on vaccination status and symptom onset.

Longitudinal cohort

157/206 (76%) individuals in the cross-sectional cohort tested with Ct-values <25 during the first three days after symptom onset. A Ct-value of <25 indicates a middle/high viral load and a relatively early state of infection, implicating relevance for transmission chains. Thus, for longitudinal analysis, only individuals with Ct-values <25 during the first three days after symptom onset were included. To ensure timely sample transportation, only individuals who lived in a radius of 3 km around the laboratory were tested.

SARS-CoV-2 detection and quantification

COBAS 6800 (Roche Diagnostics) and Alinity m (Abbott) equipped with their respective SARS-CoV-2 detection kits were used. 576 samples (96.81%) were tested on the COBAS 6800. For comparison, Ct-values measured on Alinity m were translated into copies/ml and converted into a COBAS 6800 adjusted Ct-value. To this end, seven serial dilutions (1:10) of a high titer SARS-CoV-2 sample were tested in both platforms. Using a regression model, standard curves (COBAS 6800: R^2=0.92; Alinity m: R^2=0.97) were generated (**Supplementary Fig. 1A**). Two SARS-CoV-2 samples with a quantified RNA load from INSTAND (Society for the Promotion of Quality Assurance in Medical Laboratories, e.V., Düsseldorf, Germany) were tested on each device to calculate correction factors for the standard curves. Using these correction factors, viral loads were calculated. In order to adjust the Ct-values measured on the Alinity m to the COBAS 6800, the viral load was determined and converted into a COBAS 6800 adjusted Ct-value (**Supplementary Fig. 1B-D**).

The following equation was used for calculating viral loads from Ct-values measured in COBAS 6800.

(1)

$$Viral\ load = 3E + 14 \times e^{-0.767 \times Ct}$$

Ct-values measured in Alinity m were converted into viral loads using the following equation:

(2)

$$Viral\ load = 2E + 12\ \times e^{-0.672 \times Ct}$$

Viral loads calculated from samples measured in the Alinity m were translated into COBAS 6800 adjusted Ct-values using the following equation:

(3)

$$Ct = -1.303 \ln(viral\ load) + 43.41$$

To quantify the inter-run variation of SARS-CoV-2 Ct-values, 55 Ct-values of all positive controls (derived from the same charge) tested during the observation period with COBAS 6800 were analyzed. Mean Ct-values were 32.89 (SD 0.16) and 35.41 (SD 0.39), for Orf-1a and E-Gene, respectively (**Supplementary Fig. 2**).

Analysis of the SARS-CoV-2 variant

Samples of the cross-sectional analysis were obtained from 10th of January until 21st of January 2022 and assumed to contain the BA.1 Omicron variant due to its overall predominance of all positive samples in North Rhine-Westphalia [7]. All longitudinal samples could be assigned the Omicron BA.1 VOC by genotyping (**Supplementary Table 1**). To this end, 500 µl of sample were used to purify nucleic acids with the MagNa Pure 96 automatic nucleic acid extraction instrument and the Viral NA large volume Kit (Roche Molecular Systems). 100 µl were used for elution. Of the extracted RNA, 5 µl were used in a qPCR using the VirSNip SARS-CoV-2 Spike L452R and S371L S373P assay according to the manufacturer's instructions (TIBMolBiol, Berlin, Germany). LightCycler® 480 II (Roche Diagnostics) was used for melting analysis.

Quality control of self-sampled swabs

To assess the quality of self-sampled anterior Naso-/Oropharyngeal (aNp-/Op) swabs, human β-globin-gene quantification was performed as previously described [8,9]. To this end, 53 SARS-CoV-2-negative aNp-/Op-swabs and 53 professionally attained SARS-CoV-2-negative Np-/Op-swabs from the test-center of the University Hospital Cologne were analyzed (**Supplementary Fig. 3**). There was a difference of

1.13 in mean Ct-values (32.75 vs. 33.88) for beta-Globin (p=0.0004, Mann-Whitney U-test).

Statistical analysis

Means, medians, standard deviations and 95% confidence intervals were calculated for adjusted Ct-values. Differences in Ct-values were calculated with Mann-Whitney U-test (MWU). P-values <0.05 were considered significant. Loess regression was optimized over 10,000 bootstrap runs to fit the means and 95% confidence intervals stratified by days after symptom onset (**Fig. 1B**). Matching adjusted Ct-values for confounders was done with a linear regression. Data analyses were performed using the software GraphPad Prism (v.9) and the R package stats.

Results

To investigate the viral loads during the acute phase of Omicron breakthrough infections, we analyzed data from 4,697 Np-/Op-swabs that were tested in RT-qPCR at the COVID-19 test center of the University Hospital Cologne during the period 10th of January until 21st of January, 2022. 3,714 swabs (79.1%) were obtained from employees and 983 (20.9%) from outpatients. 468 individuals tested positive. 100 (21.3%) were excluded due to a positive result before the observation period. The remaining 368 individuals were categorized as symptomatic, if at least one COVID-19 associated symptom was newly reported within 7 days prior to or after diagnosis. COVID-19 associated symptoms included fever, cough, sore throat, headache, rhinitis, nausea, diarrhea, shortness of breath, myalgia, loss of taste and/or loss of smell. 284 (77.1%) individuals had symptoms, 26 (7.1%) reported no symptoms. Data on symptoms were not available for 58 (15.8%) individuals. Of the symptomatic individuals, 156 (54.9%) were boostered and 110 (38.7%) were double vaccinated at

least 14 days before infection. Of 18 (6.4%), the vaccination status was either not available or the individuals reported past infections. Of the 266 symptomatic infected individuals that were boostered or double vaccinated, 206 (77.4%) were within the first three days after symptom onset at the day of sample collection. Of those, the median age was 29.5 years (range, 18-67 years) and gender was nearly equally distributed (49% female, 51% male) (**Table 1A**). Ct-values were determined in a routine diagnostic setting by RT-qPCR. The mean of the Ct-values detected in double vaccinated individuals (mean: 21.76; CI: 20.87-22.64) was significantly lower than in boostered individuals (mean 23.14; CI: 22.35-23.94; p=0.029, Mann-Whitney U-test; **Fig. 1A**). Corresponding geometric means of viral loads were $1.66x10^7$ and $5.74x10^6$ SARS-CoV-2 copies/ml (95% CI: $6.71x10^6$-$1.54x10^8$ and $6.48x10^5$-$7.01x10^7$). Moreover, 83.72% of the double vaccinated individuals and 70.83% of the boostered individuals had a fraction of Ct-values <25 (viral load >$1.38x10^6$ copies/ml) within the first three days after symptom onset (**Fig. 1A**). These results did not change after matching for age, gender and days after symptom onset. We conclude that booster immunization leads to a decrease of peak viral loads in Omicron breakthrough infections.

In correspondence to the viral loads of the majority of the cross-sectional cohort (**Fig. 1A**), for longitudinal analysis, only individuals with middle/high viral loads (at least one Ct-value <25 during the first three days after symptom onset) were tested. 59.3% of the individuals were female and 40.7% were male. Median age was 29 years (range, 18-64 years). 10 (31.2%) individuals were double vaccinated and 22 (68.8%) were boostered. Of the double vaccinated individuals, median time between second dose of vaccination and positive RT-qPCR was 160 days (range, 83-309 days). Of the boostered individuals, median time between third dose of vaccination and positive RT-qPCR was 31 days (range, 13-91 days; **Table 1B**).

In total, 389 samples of 32 individuals were analyzed (mean, 11.7 swabs per individual; **Fig.1B**). The mean Ct-values of double vaccinated and boostered individuals increased from 22.9 and 21.8 on day 0 after symptom onset, respectively, to a threshold of 30 ($2.96x10^4$ copies/ml) after 8 and 9 days, respectively (**Fig. 1B**). On day 9 after symptom onset, of the double vaccinated individuals, 79.4%, 99.0%, and 100.0% were detected with Ct-values ≥30, ≥25, and ≥20, respectively (≥$2.96x10^4$, ≥$1.38x10^6$, ≥$2.96x10^4$ copies/ml). Of the boostered individuals, 54.2%, 89.3%, and 99.5% were detected with Ct-values ≥30, ≥25, and ≥20, respectively. On day 14 after symptom onset, mean Ct-value was 37.7 ($7.99x10^1$ copies/ml) for double vaccinated and 38.5 ($4.32x10^1$ copies/ml) for boostered individuals. (**Fig. 1B**). We conclude that there are no significant differences in viral load trajectory between double vaccinated and boostered individuals with Omicron breakthrough infection if the initial viral load is similar in both groups.

Our data show that a booster vaccination reduces the viral load during the early phase of infection with the Omicron variant compared to a double vaccination, but does not shorten the duration of viral clearance.

Discussion

Epidemiological data show a reduced transmissibility for the Omicron VOC in boostered compared to double vaccinated individuals [2] and infectious viral loads were shown to be decreased by a booster vaccination [10]. In Delta infections, the viral load in double vaccinated is reduced in comparison to that in unvaccinated individuals. This effect is absent after 120 days, but can be temporarily restored by a booster vaccination[6]. Nevertheless, a follow-up study by Levine-Tiefenbruch et al. [11] shows that the restored effect by the booster vaccination also wanes and vanishes after 60 days. While our study shows that booster vaccination reduces peak

viral loads, it does not answer if this effect is maintained over time in Omicron breakthrough infections and thus, follow-up studies would be required.

Viral clearance in breakthrough infections is faster in immunized individuals than in unvaccinated infected with variants other than the Omicron VOC [12,13]. However, we did not observe an analog effect in boostered vs double immunized individuals with Omicron breakthrough infections with similar initial viral loads which is in line with a study by Puhach et al. [10]. In accordance with that study, there might be a difference in infectious viral loads rather than between the duration of viral shedding between the two groups of our study.

Limitations of this study include a small sample and a selection-bias towards lower initial Ct-values in the longitudinal cohort. However, these individuals represent the vast majority of newly diagnosed cases as observed in the cross-sectional cohort. Another limitation is the specimen quality due to the self-sampling of the longitudinally tested individuals. However, we consider the determined systematic difference of 1.13 Ct-values for the human ß-Globin-gene between professional sampling and self-sampling as admissible for the conclusions drawn from the data in this study. The composition of both cohorts is not representative for the general population with a selective distortion towards generally younger individuals. Because the duration of viral shedding is positively associated with age [14], our study might underestimate the duration of viral shedding in Omicron breakthrough infections. Nevertheless, despite an age-dependency in viral shedding and a possible underestimation in our study, viable SARS-CoV-2 was shown to be short-lived [14], limiting the relevance of the age of the individuals of this study for the infection dynamics in the population. Lastly, the limited availability of clinical data is one limitation that is due to the retrospective nature of our study.

Data availability

All shown data will be made available on request.

Author contributions

Conceptualization, F.D., S.D. and F.K; methodology, F.D. and S.D.; investigation, F.D., S.D. and M.H.; resources, E.H., F.K. and J.Z; formal analysis, F.D., S.D., M.P., M. Hellmich and F. Klein; writing—original draft, F.D. and S.D.; writing—review and editing, all authors; visualization, F.D., S.D. and F.K; supervision, F. K. and J.Z.

Funding

There is no external funding.

Acknowledgements

We thank all participants taking part in our investigation. We thank all members of the Institute of Virology, University Hospital Cologne. In particular, we thank Karin Decker, Magarete Windelschmidt, Irina Fish, Ivonne Torre-Lage, Christina Hellriegel and Esther Milz for processing SARS-CoV-2 samples. We also thank all members of "Bike Syndikat Kurierdienst GbR" for sample transportation.

Competing interest declaration

The authors have no competing interests.

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Figure legends

Fig. 1: Viral load kinetics in Omicron breakthrough-infections

a, Viral load of Omicron breakthrough-infections was determined during day 0-3 after symptom onset. The 206 samples are plotted by adjusted Ct-value and stratified by vaccination status ($p<0.029$, MWT). Bars and numbers above indicate mean Ct-values (left y-axis) or geometric mean viral load (right y-axis). Fractions of Ct-values <25 or ≥25 are indicated by corresponding colors and the absolute numbers are given in the pie charts. **b**, Vaccinated individuals with Omicron breakthrough-infections obtained self-sampled aNp-/Op-swabs daily until 14 days after symptom onset. Vaccination status is indicated by corresponding colors. Adjusted Ct-values are plotted longitudinally and stratified by days after symptom onset (left panels). Dashed lines indicate the limit of detection (left) or a Ct-value of 30 (right), respectively. Loess regression was optimized for fitting of the means and is stratified by days after symptom onset. 95% confidence intervals are indicated by corresponding colored areas. Fractions of individuals with adjusted Ct-values ≥ 30, 25 or 20 are stratified by days after symptom onset and indicated by corresponding colored lines.

Table 1: Participants demographics

A

Cross-sectional Omicron breakthrough infection cohort

Infected individuals at the COVID-19 test center n=368			n	368
	General information	*Citizens*	n (%)	278 (75.6%)
		Employees	n (%)	90 (24.4%)
	Symptoms	*Symptomatic*	n (%)	284 (77.1%)
		Asymptomatic	n (%)	26 (7.1%)
		NA	n (%)	58 (15.8%)
Symptomatic individuals n=284			n	284
	Days after symptom onset	*0-3 days*	n (%)	215 (75.7%)
		>3 days	n (%)	69 (24.3%)
	Immune status	*Double vaccinated*	n (%)	110 (38.7%)
		Boostered	n (%)	156 (54.9%)
		Other	n (%)	18 (6.3%)
Participants n=206			n	206
	Gender	*Female*	n (%)	101 (49%)
		Male	n (%)	105 (51%)
	Age		Median years (range)	29.5 (18-67)
	Vaccination status	*Double vaccinated*	n (%)	86 (41.7%)
		Boostered	n (%)	120 (58.3%)

B

Longitudinal Omicron breakthrough cohort

Participants n=32			*n*	32
	Gender	*Female*	*n (%)*	19 (59.3%)
		Male	*n (%)*	13 (40.7%)
	Age		*Median years (range)*	29 (18-64)
	Vaccination status	*Double vaccinated*	*n (%)*	10 (31.2%)
		Days between second dose and first positive RT-qPCR	*Median days (range)*	160.5 (89-309)
		Boostered	*n (%)*	22 (68.8%)
		Days between third dose and first positive RT-qPCR	*Median days (range)*	31 (13-91)

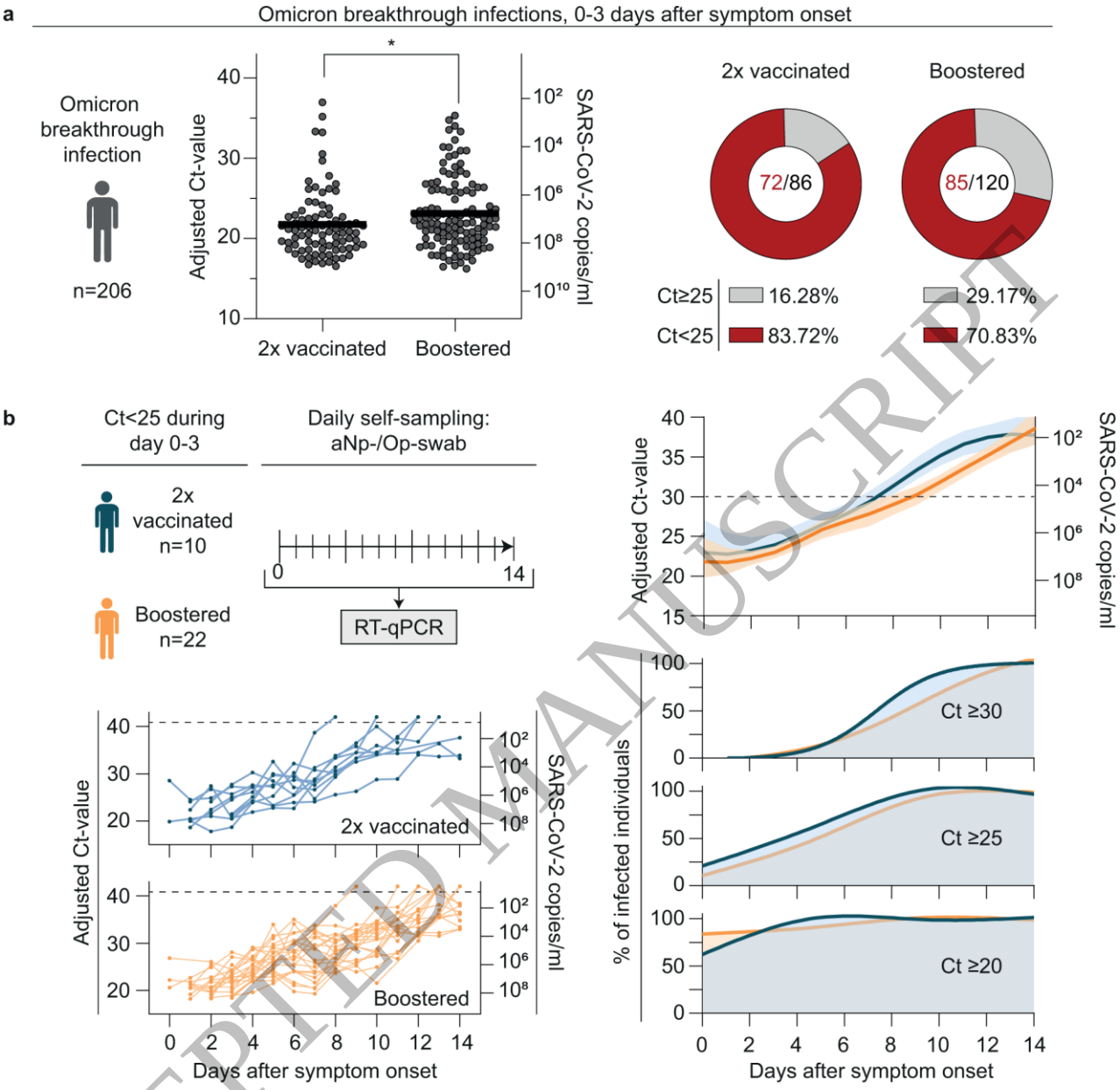

Figure 1
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