Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Research note

RNA loads of severe acute respiratory syndrome coronavirus 2 in patients with breakthrough coronavirus disease 2019 caused by the Delta and Omicron variants

Paula de Michelena 1, Ignacio Torres 1, Enric-Cuevas Ferrando 2, Beatriz Olea 1, Fernando González-Candelas 3, Gloria Sánchez 2, David Navarro 1, 4,*

1 Microbiology Service, Clinic University Hospital, Instituto de Investigación Sanitaria Hospital Clínico Valencia Health Research Institute, Valencia, Spain
2 Department of Preservation and Food Safety Technologies, Institute of Agrochemistry and Food Technology, Instituto de Agroquímica y Tecnología de Alimentos-Centro Superior de Investigaciones Científicas, Valencia, Spain
3 Joint Research Unit Infection and Public Health Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana-University of Valencia, Institute for Integrative Systems Biology (I2SysBio, Universitat de València-Centro Superior de Investigaciones Científicas) and Centro de Investigación Biomédica en Red en Epidemiology and Public Health, Valencia, Spain
4 Department of Microbiology, School of Medicine, University of Valencia, Valencia, Spain

A R T I C L E   I N F O

Article history:
Received 25 March 2022
Received in revised form 31 August 2022
Accepted 7 September 2022
Available online 15 September 2022
Editor: M. Cevik

Keywords:
COVID-19
Delta variant
Infectious viral load
Omicron variant
SARS-CoV-2
SARS-CoV-2 RNA load
Viability RT-PCR assay

A B S T R A C T

Objectives: To compare the RNA loads of severe acute respiratory syndrome coronavirus 2 in nasopharyngeal specimens collected from patients with breakthrough coronavirus disease 2019 (COVID-19) caused by the Delta variant with those in specimens collected from patients with breakthrough COVID-19 caused by the Omicron variant.

Methods: A retrospective, observational study was conducted, including 240 consecutive adult outpatients, of whom 121 (74 females; median age, 40 years) had COVID-19 due to the Omicron variant and 119 (65 females; median age, 48 years) had COVID-19 caused by the Delta variant. The viral RNA load was quantitated using the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Waltham, MS, USA).

The viability platinum chloride reverse transcription-PCR assay was used to discriminate between potentially infectious viral particles and free (encapsidated) viral RNA.

Results: Overall, the viral RNA loads were significantly higher (p 0.003) for the Omicron variant (median, 8.1 log10 copies/mL; range, 4.0–10.9 log10 copies/mL) than for the Delta variant (median, 7.5 log10 copies/mL; range, 3.0–11.6 log10 copies/mL). A trend towards higher viral loads was noticed for Omicron compared with that for Delta across the following time frames since vaccination: 16–90 days (median, 6.83 vs. 5.88 log10 copies/mL, respectively; range, 3.91–10.68 vs. 3.67–9.66 log10 copies/mL, respectively; p 0.10), 91–180 days (median, 8.09 vs. 7.46 log10 copies/mL, respectively; range, 4.30–10.92 vs. 3.03–11.56 log10 copies/mL, respectively; p 0.003) and 181–330 days (median, 8.56 vs. 8.10 log10 copies/mL, respectively; range, 6.51–10.29 vs. 3.03–10.61 log10 copies/mL; p 0.11). The platinum chloride treated or untreated reverse transcription-PCR cycle threshold ratio for the nucleocapsid gene as the target was slightly higher for Omicron than for Delta (median, 0.62 vs. 0.57, respectively; range, 0.57–0.98 vs. 0.61–0.87, respectively), although statistical significance was not reached (p 0.10).

Conclusion: The time elapsed since vaccination has a major impact on the RNA loads of severe acute respiratory syndrome coronavirus 2 in nasopharyngeal specimens, particularly for the Omicron variant. The Omicron variant may be better adapted for replication in the upper respiratory tract than the Delta variant, in which this is unlikely given its more efficient generation of viral particles. Paula de Michelena, Clin Microbiol Infect 2023;29:256.e1–256.e4

© 2022 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

* Corresponding author. David Navarro, Microbiology Service, Hospital Clínico Universitario, Instituto de Investigación Sanitaria Hospital Clínico Valencia, Av. Blasco Ibáñez 17, 46010 Valencia, Spain.
E-mail address: david.navarro@uv.es (D. Navarro).

https://doi.org/10.1016/j.cmi.2022.09.003
1198-743X/© 2022 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.
Introduction

The new variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron (BA.1.1.529) was first reported in South Africa in November 2021 [1] and has spread rapidly since then because of its seemingly increased transmissibility [2,3] to become dominant in many countries. Evasion from spike (S)-binding neutralizing antibodies elicited during natural infections or after vaccination may account for the transmission advantage of the Omicron variant over the Delta variant [4–8]. Omicron has seven amino acid deletions across the N-terminal domain of S and approximately 30 substitutions within the S-receptor-binding domain, some of which are critically involved in neutralizing antibody binding [9] (Table S1). Nevertheless, increased replication efficiency in the upper respiratory tract, which could translate into higher viral loads, may also contribute to increased transmissibility; in fact, the Omicron variant is characterized by a total of ten amino acid substitutions located within the receptor-binding motif, which may affect the affinity of SARS-CoV-2 for the angiotensin-converting enzyme 2 receptor [9–11]. We have previously shown that the time elapsed since vaccination exerts a major impact on the magnitude of the RNA loads of SARS-CoV-2 in nasopharyngeal specimens collected from patients with the Delta variant [12]. A similar observation was made for the Omicron variant [13]. Here, we compared both the whole RNA loads of SARS-CoV-2 and potentially infectious virus in nasopharyngeal specimens collected from patients with breakthrough coronavirus disease 2019 (COVID-19) caused by the Delta variant and those in specimens collected from patients with breakthrough COVID-19 caused by the Omicron variant; the latter was detected using the viability reverse transcription (RT)-PCR assay, which can discriminate between potentially infectious viral particles and free (encapsidated) viral RNA [14].

Methods

Patients

We conducted a retrospective, observational study, including 240 consecutive adult out-patients (age ≥ 16 years) who visited the Health Department of Clínico-Malvarrosa (Valencia, Spain) between September 2021 and February 2022, of whom 121 (74 females; median age, 40 years; range, 16–101 years) and 119 (65 females; median age, 48; range, 16–89 years) developed breakthrough symptomatic SARS-CoV-2 infection due to the Omicron and Delta variants, respectively. Breakthrough symptomatic infection was defined as that developing at least 15 days after the receipt of the last vaccination dose. Data regarding signs or symptoms potentially associated with COVID-19, such as fever, cough, rhinorrhea, odynophagia, cephalea, myalgia, dyspnoea, ageusia and anosmia, obtained from the patients’ medical records were used to establish the time since the onset of the symptoms (any of the above). The study was approved by the Instituto de Investigación Sanitaria Hospital Clínico de Valencia Research Ethics Committee (2021/165), and informed consent was waived because of its retrospective nature.

RT-PCR assays

The RNA loads of SARS-CoV-2 in nasopharyngeal specimens were estimated using the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Waltham, MS, USA), which was calibrated to the AMPLICR® TOTAL SARS-CoV-2 RNA Control (Vircell SA, Granada, Spain) [12]. The involvement of the Delta variant was documented using either whole-genome sequencing or variant-specific RT-PCR (SARS-CoV-2 PCR variant, Ascires, Sistemas Genómicos, Valencia, Spain) [12]. The Omicron variant was identified either using whole-genome sequencing or inferred based on S-gene target failure in RT-PCR using the Thermo Fisher kit. The viability RT-PCR assay was performed as previously described [14]. Briefly, 300 μL of nasopharyngeal specimens was pre-treated or mock treated with 2.5 mM platinum chloride, which precludes the amplification of naked viral RNA, in DNA in LoBind tubes (Eppendorf, Hamburg, Germany) for 30 minutes at room temperature in an orbital shaker (150 rpm). Viral RNA was extracted using the KingFisher™ Flex platform (Thermo Fisher Scientific) and detected using quantitative RT-PCR, which targeted the nucleocapsid (N) gene sequence. Each quantitative RT-PCR assay was performed in duplicate and included nuclease-free water as a negative control. The data are reported as the ratio of RT-PCR cycle thresholds ($C_T$) for the N amplicon returned by the pre-treated or untreated specimens.

Statistical analysis

The medians of continuous variables were compared using the Mann-Whitney U test or Kruskal-Wallis test, as appropriate. Frequency comparison between the groups was performed using the Fisher exact test. Two-sided exact p values were reported. A p value of <0.05 was considered statistically significant. The analyses were performed using SPSS, version 20.0 (SPSS, Chicago, IL).

Results

A unique nasopharyngeal specimen was collected from 240 individuals with symptomatic SARS-CoV-2 infection due to either the Delta or Omicron variant at the time the patients sought medical assistance (a median of 2 days after the onset of the symptoms; range, 0–7 days in both the study groups). Overall, the viral RNA loads were significantly higher (p 0.003) for the Omicron variant (median, 8.1 log10 copies/mL; range, 4.0–10.9 log10 copies/mL) than for the Delta variant (median, 7.5 log10 copies/mL; range, 3.0–11.6 log10 copies/mL). As shown in Table 1, patients in the two groups were comparable for the vaccination schedule employed, the median time elapsed since the receipt of the last vaccination dose (134 days [range, 16–324 days] for Omicron and 158 days [range, 20–318 days] for Delta; p 0.13) and SARS-CoV-2 infection prior to vaccination.

To assess the impact of the time elapsed since the completion of vaccination on the RNA loads of SARS-CoV-2 Omicron and Delta in the nasopharyngeal specimens, we arbitrarily defined the following time frames: 16–90, 91–180 and >180 days. The specimens were collected at comparable times from the time of the onset of the symptoms across these time windows for both cases of the Omicron and Delta variants. As shown in Fig. 1, higher viral RNA loads were observed to be associated with the time from vaccination for both the variants, most notably for Omicron. Higher viral RNA loads were noticed for Omicron than for Delta across all the periods: 16–90 days (median, 6.83 vs. 5.88 log10 copies/mL; range, 3.91–10.68 vs. 3.67–9.66 log10 copies/mL; p 0.10), 91–180 days (median, 8.09 vs. 7.46 log10 copies/mL; range, 4.30–10.92 vs. 3.03–11.56 log10 copies/mL; p 0.003) and 181–330 days (median, 8.56 vs. 8.10 log10 copies/mL; range, 6.51–10.29 vs. 3.03–10.61 log10 copies/mL; p 0.11).

To assess the relative proportion of free viral RNA in the nasopharyngeal specimens, a total of 17 and 15 samples from patients with breakthrough COVID-19 caused by the Omicron and Delta variants, respectively, were subjected to the viability RT-PCR assay (Table S2). The platinum chloride treated or untreated RT-PCR $C_T$
ratio for the N target was higher for Omicron than for Delta in two different experiments (mean, 0.62 vs. 0.57, respectively; range, 0.57–0.98 vs. 0.61–0.87, respectively), although statistical significance was not reached (p 0.10).

**Discussion**

Several key observations were made in the present study. First, the RNA loads of SARS-CoV-2 Omicron and Delta in the nasopharyngeal specimens were found to increase in individuals with breakthrough COVID-19 with time elapsed since the receipt of the last vaccination dose. This supports the notion that waning vaccine-elicited immunity has a major impact on the RNA loads of SARS-CoV-2 in the upper respiratory tract, which is in agreement with previously published data on the Delta variant [12,13]. The size of the effect was more marked for the Omicron variant, likely reflecting its advantage over the Delta variant in evasion from vaccine-induced immune responses [4–8]. Second, the RNA loads of SARS-CoV-2 Omicron tended to be higher than those of Delta regardless of the time elapsed since vaccination, although a statistically significant difference was only seen between days 91 and 180; we speculated that the former variant may outperform...
the latter in terms of replicative efficiency in the upper respiratory tract, which is in concordance with previous assumptions [15]. Nevertheless, our observation must be interpreted with caution because peak viral load may occur earlier in Delta infections than in Omicron infections [16]. In contrast to our results, Migueres et al. [7] reported higher RNA loads for Delta than for Omicron in vaccinated participants, although they did not disclose whether the comparison groups were matched for the time passed since vaccination.

In conclusion, we provide evidence highlighting the impact of Delta for Omicron in vaccinated participants, although they did not disclose whether the comparison groups were matched for the time passed since vaccination. In turn, Puhach et al. [8] found comparable viral RNA loads (E-gene Ct values) for both the variants in individuals who experienced SARS-COV-2 infection a mean of approximately 5 months after vaccination, in line with our observations. Third, the viability RT-PCR assay revealed no evidence of a higher infectious RNA load in the nasopharyngeal specimens collected from patients infected with the Omicron or Delta variant, which concur with data from a previous study that used a virus-induced, focus-forming assay [8].

The current study has several limitations. First, we could not rule out differences in quality (cellularity) across the specimens from the patients in the comparison groups, which may have impacted the measured viral RNA loads. Second, no viral cultures were performed to evaluate the content of viable viral particles in the nasopharyngeal specimens collected from the patients in the two groups. Third, no data on patient comorbidities, which could also have impacted the magnitude of viral RNA loads in the upper respiratory tract [17], were available.

In conclusion, we provide evidence highlighting the impact of time elapsed since vaccination on the RNA loads of SARS-COV-2 in nasopharyngeal specimens, particularly when the Omicron variant is involved. Moreover, the data indirectly suggested that the Omicron variant is better adapted for replication in the upper respiratory tract than the Delta variant, thereby generating higher viral loads.

Author contributions

PdM, IT, EC-F and FG-C: methodology and data validation. GS and DN: study design and logistics; DN: conceptualization, data analysis and manuscript writing.

Transparency declaration

IT holds a Río Hortega Contract (CM20/00090) funded by the Carlos III Health Institute (co-financed by the European Regional Development Fund, ERDF/ERDF/FEDER). EC-F holds a pre-doctoral contract from MICINN (2018).

Acknowledgements

We are grateful to the residents and staff at the Microbiology Service of Hospital Clínico Universitario and the medical and nursing staff at the primary health centres of the Health Department of Clínico-Malvarrosa.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcmi.2022.09.003.

References

[1] World Health Organization. Classification of Omicron (B.1.1.529): SARS-Cov-2 variant of concern. https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern.
[2] Espenhamn Í, Funk T, Overvad M, Edslev SM, Fonager J, Ingham AC, et al. Epidemiological characteristics of the first 785 SARS-CoV-2 Omicron variant cases in Denmark, December 2021. Euro Surveill 2021;26:2101146. https://doi.org/10.2807/1560-7917.EU.2021.26.50.2101146.
[3] Andrews N, Stowe J, Kirsebom F, Toffa S, Rickeard T, Gallagher E, et al. Covid-19 vaccine effectiveness against the Omicron (B.1.1.529) variant. N Engl J Med 2022;386:1532–46. https://doi.org/10.1056/NEJMoa22119451.
[4] Hoffmann M, Krüger N, Schulz S, Cossmann A, Rocha C, Kempf A, et al. The Omicron variant is highly resistant against antibody-mediated neutralization: implications for control of the COVID-19 pandemic. Cell 2022;185:447–56. https://doi.org/10.1016/j.cell.2022.12.032.
[5] Cele S, Jackson L, Khoury DS, Khan K, Moyo-Gwete T, Tegally H, et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. Nature 2022;592:654–6. https://doi.org/10.1038/s41586-021-04257-1.
[6] Grabowski F, Kochanekcz M, Lipniacki T. The spread of SARS-CoV-2 variant Omicron with a doubling time of 2.0–3.3 days can be explained by immune evasion. Viruses 2022;14:294. https://doi.org/10.3390/v14020294.
[7] Migueres M, Dinmeglio C, Trémaux P, Abravanel F, Raymond S, Lhomme S, et al. Influence of immune escape and nasopharyngeal virus load on the spread of SARS-CoV-2 Omicron variant. J Infect 2022;84:834–72. https://doi.org/10.1007/s11586-021-01386-3.
[8] Puhach O, Adea K, Hulo N, Satronnet P, Genecaud C, Iren A, et al. Infectious viral load in unvaccinated and vaccinated patients infected with SARS-CoV-2 WT, Delta and Omicron. Nat Med 2022;28:1491–500. https://doi.org/10.1016/j.nmed.2022.01.010.
[9] Jung C, Kmiec D, Koepke L, Zech F, Jacob T, S Parrer KM, et al. Omicron: what makes the latest SARS-CoV-2 variant of concern so concerning? J Virol 2022;96:e02077231. https://doi.org/10.1128/jvi.02077-21.
[10] Rath SL, Padhi AK, Mandal N. Scanning the RBD-AE2 molecular interactions in Omicron variant. Biochim Biophys Res Commun 2022;592:18–23. https://doi.org/10.1016/j.bbrc.2022.01.006.
[11] Wu L, Zhou L, Mo M, Liu T, Wu C, Gong C, et al. SARS-CoV-2 Omicron RBD shows weaker binding affinity than the currently dominant Delta variant to human ACE2. Signal Transduct Target Ther 2022;7:38. https://doi.org/10.1038/s41392-021-00863-2.
[12] de Michelen P, Torres J, Albert E, Bracho A, González-Candelas F, Navarro D. Impact of time elapsed since full vaccination on SARS-CoV-2 RNA load in Delta-variant breakthrough COVID-19. J Infect 2022;84:579–613. https://doi.org/10.1007/s11586-021-01386-3.
[13] Levine-Tiefenbrun M, Yelin I, Alapi H, Herzl E, Kunt J, Chodick G, et al. Waning of SARS-CoV-2 booster viral-load reduction effectiveness. Nat Commun 2022;13:1237. https://doi.org/10.1038/s41467-022-28936-y.
[14] Cuevas-Ferrando E, Randazzo W, Pádua-Silva L, Falcón I, Navarro D, Martin-Latif S, et al. Platinum chloride-based viability RT-qPCR for SARS-CoV-2 detection in complex samples. Sci Rep 2021;11:18120. https://doi.org/10.1038/s41598-021-97710-x.
[15] Peacock TP, Brown JC, Zhou J, Thakur N, Newman J, Kugathasan R, et al. The Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. Lancet Infect Dis 2022;22:183–95. https://doi.org/10.1016/S1473-3099(21)00648-4.
[16] Maltezou HC, Raffopoulos V, Voros R, Papadima K, Mellou K, Spanakis N, et al. Association between upper respiratory tract viral load, comorbidities, disease severity, and outcome of patients with SARS-CoV-2 infection. J Infect Dis 2023;213:1132–8. https://doi.org/10.1093/infdis/jiaa804.

https://doi.org/10.1016/j.cmi.2022.09.003.