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CLINICAL MICROBIOLOGY - SHORT COMMUNICATION

COVID-19 in immunocompromised children: comparison of SARS-CoV-2 viral load dynamics between the first and third waves

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Abstract

SARS-CoV-2 dynamics across different COVID-19 waves has been unclear in immunocompromised children. We aimed to compare the dynamics of SARS-CoV-2 RNA viral load (VL) during the first and third waves of COVID-19 in immunocompromised children. A retrospective and longitudinal cohort study was conducted in a pediatric referral hospital of Argentina. The study included 28 admitted immunocompromised children with laboratory confirmed SARS-CoV-2 infection. Thirteen acquired the infection during COVID-19 first wave (May to August 2020, group 1 (G1)) and fifteen in the third wave (January to March 2022, group 2 (G2)). RNA viral load measure and its dynamic reconstruction were performed in nasopharyngeal swabs by validated quantitative, real time RT-PCR, and linear mixed-effects model, respectively. Of the 28 children included, 54% were girls, most of them had hemato-oncological pathology (57%), and the median age was 8 years (interquartile range (IQR): 3–13). The dynamic of VL was similar in both groups ($P=0.148$), starting from a level of 5.34 $\log_{10}$ copies/mL (95% confidence interval (CI): 4.47–6.21) in G1 and 5.79 $\log_{10}$ copies/mL (95% CI: 4.93–6.65) in G2. Then, VL decayed with a rate of 0.059 (95% CI: 0.038–0.080) and 0.088 (95% CI: 0.058–0.118) $\log_{10}$ copies/mL per day since diagnosis and fell below the limit of quantification at days 51 and 39 after diagnosis in G1 and G2, respectively. Our results evidenced a longer viral RNA persistence in immunocompromised pediatric patients and no difference in VL dynamic between COVID-19 first wave—attributed to ancestral infections—and third wave—attributed to Omicron infections.

Keywords COVID-19 · Immunocompromised patient · Pediatrics · Population dynamics · Viral load

Introduction

The viral dynamics of SARS-CoV-2 is of paramount importance for the epidemiology of COVID-19 and infection control policies. The duration of test positivity influences infectiousness [1, 2] and isolation policies, test recommendations, and clinical care guidelines [3, 4]. Evidence on the persistence of SARS-CoV-2 in pediatric immunocompromised patients has not been well established and gain relevance. There have been several case reports that found infectious SARS-CoV-2 beyond 20 days since symptoms onset in immunocompromised patients [5–11]. This prolonged duration of infection could accelerate the emergence and spread of new variants [12–14]. Nevertheless, RNA viral load (VL) data in this population is limited and even more in immunocompromised pediatric patients. Also, most of the reports were conducted during the first two waves of COVID-19, and it is unknown whether new variants of SARS-CoV-2 favor prolonged VL and increase the risk of severe COVID-19 in immunocompromised pediatric patients.

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In Argentina, first wave began during the end of autumn (March 2020) and ended at winter (August 2020). At that moment, most SARS-CoV-2 infections belonged to ancestral linage [15]. By the beginning of 2022 (in the middle of the summer), Argentina experienced their third wave of COVID-19, with absolutely predominance of Omicron variant (> 98% from the first week of January 2022) [16]. The aim of this study was to compare the dynamics of VL between immunocompromised infants, and children get infected during first and third waves of COVID-19.

Methods

Study design and population

This was a single-center, retrospective, and longitudinal cohort study conducted at the Hospital de Pediatría Garrahan, the major high complexity referral pediatric hospital in Buenos Aires, Argentina. The study included all the admitted immunocompromised patients having (i) SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) positive from May to August 2020 (COVID-19 first wave) or from January to March 2022 (COVID-19 third wave), (ii) age between 0 and 18 years old, and (iii) at least three nasopharyngeal swab samples available throughout two or more weeks since SARS-CoV-2 diagnosis. Out of 2,915 children and adolescents tested against SARS-CoV-2 during the first wave, 193 were positive (7%), and of them, thirteen met all the inclusion criteria. While, during the third wave 10,752, children and adolescents were tested, 2,130 resulted positive (20%), and fifteen of them were included in the study. Patients were stratified according to the wave they acquired SARS-CoV-2 infection: group 1 (G1) for the first wave and group 2 (G2) for the third one.

This study was approved by the Institutional Review Board (Comité Revisor y de Ética en la Investigación, Hospital de Pediatría "Prof. Dr. Juan P. Garrahan" Protocol No. 1359), and written informed consent was obtained from parents or legal guardians.

RNA viral load of SARS-CoV-2

Viral RNA extraction and genomic VL were performed as previously described [17]. Briefly, RNA was isolated from 500 µL of nasopharyngeal swabs using the automated MagNA Pure 96 DNA and viral NA large volume kit (Roche, Germany), within 24 h since the sample was collected. The SARS-CoV-2 VL was measured by a quantitative, real-time RT-PCR targeting a region of the N gene of SARS-CoV-2 on a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems). The assay had an efficacy of 99%, a specificity of 100%, a repeatability of 2.31% (coefficient of variation), and a dynamic range from 10 to 1×10^8 copies per reaction (equivalent to a range from 400 to 4×10^9 copies per mL).

Statistical analysis

The trajectories of SARS-CoV-2 VL during the first and third waves of COVID-19 were estimated by a linear mixed-effects (LME) model with a random slope and intercept for each wave. Gender, age, and COVID-19 severity were tested to estimate the VL trajectories, but none of them improve the model fit—based on the minimization of the Akaike information criterion—and were therefore not included in the final model. The Kaplan–Meier plot with a log-rank test was used to assess differences in viral RNA clearance between waves. To analyze differences between the first and third wave for categorical and continuous variables, Fisher and Mann–Whitney–Wilcoxon tests were performed, respectively. Pearson's correlation coefficient was used to evaluate the linear relationship between SARS-CoV-2 VL values and cycle threshold (C_T) values for N gene obtained by Food and Drug Administration approved molecular diagnostic tests for SARS-CoV-2 (RealStar® SARS-CoV-2 RT-PCR Kit; GeneFinder™ COVID-19 Plus RealAmp Kit; and PerkinElmer® SARS-CoV-2 real-time RT-PCR assay, and the assay developed by the Institute of Virology Charité Universitätsmedizin). Statistical analyses were performed with R, version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

Among the 28 children included in the study, the median age was 8 years [interquartile range (IQR): 3–13]. Of them, thirteen acquired SARS-CoV-2 infection during the first wave (G1) and fifteen during the third wave (G2) of COVID-19. Demographic data and clinical characteristics of the patients included in each group are presented in Table 1. The underlying pathology was hemato-oncological in most cases (57%), followed by solid tumor (18%), other immunocompromising condition (14%), pediatric solid organ (SOT, 7%), and hematopoietic stem cell transplants (HSCT, 4%). Regarding the severity of the COVID-19, most children experienced a mild disease (57%) or were asymptomatic (32%), and only three had a severe or critical evolution (11%). Three patients of G2 had received at least one dose of SARS-CoV-2 vaccine. Two of them received one dose of Pfizer-BioNTech vaccine and the other one received two doses of Sinopharm. A difference in the gender distribution was observed between G1 and G2, with a predominance of girls in G2 ($P < 0.01$). The number of samples measured per patient was similar in both groups, with a median of 6 (IQR): 5–7) for G1 and 7 (IQR: 6–7) for G2. Also, the period

Table 1 Clinical characteristics of immunocompromised children

Variable	Total ($n=28$)	Group 1 ($n=13$)	Group 2 ($n=15$)	p value
Gender, no. (%)				<0,01†
Girls	15 (53,6)	3 (23,1)	12 (80,0)	
Boys	13 (46,4)	10 (76,9)	3 (20,0)	
Age, y median (IQR)	8 (3–13)	13 (5–14)	6 (2–10)	0,087‡
Immunocompromising condition, no. (%)				0,021†
Oncology—solid tumor	5 (17,9)	3 (23,0)	2 (13,3)	
Oncology—leukemia/lymphoma	16 (57,1)	4 (30,8)	12 (80,0)	
HSCT	1 (3,6)	1 (7,7)	0 (0,0)	
SOT	2 (7,1)	1 (7,7)	1 (6,7)	
Other	4 (14,3)	4 (30,8)	0 (0,0)	
COVID-19 severity, no. (%)				0,197†
Asymptomatic	9 (32,1)	4 (30,8)	5 (33,3)	
Mild	16 (57,2)	6 (46,1)	10 (66,7)	
Severe/critic	3 (10,7)	3 (23.1)	0 (0,0)	

HSCT, hematopoietic stem cell transplant; *SOT*, solid organ transplant. †Fisher test. ‡Mann–Whitney test

of study was similar between both groups: a median of 46 (IQR: 30–69) days since diagnosis for G1 and a median of 50 (IQR: 45–61) days since diagnosis for G2.

RNA viral load dynamics of SARS-CoV-2

The LME model showed an overlap between the trajectories of G1 and G2, evidencing no difference in the dynamic of SARS-CoV-2 RNA VL by the wave of COVID-19 (Fig. 1). At the moment of SARS-CoV-2 diagnosis, RNA VL was 5.34 (95% confidence interval (CI): 4.47–6.21] $\log_{10}$ copies/mL in G1 and 5.79 (95% CI: 4.93–6.65) $\log_{10}$ copies/mL in G2. Then, RNA VL decayed with a rate of 0.059 (95% CI: 0.038–0.080) $\log_{10}$ copies/mL per day since diagnosis in G1 and 0.088 (95% CI: 0.058–0.118) $\log_{10}$ copies/mL per day since diagnosis in G2. It is important to note that the RNA VL dynamic was not affected by the age ($P=0.966$), sex ($P=0.176$), and severity of COVID-19 ($P=0.188$). Next, we

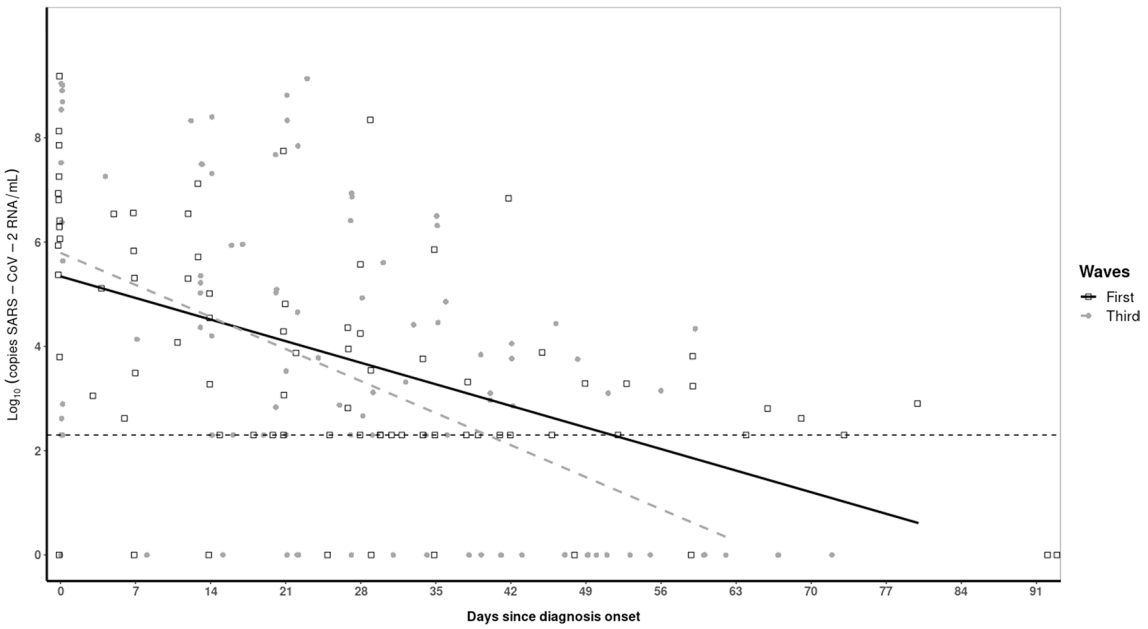

Fig. 1 Comparison of SARS-CoV-2 RNA VL dynamics in nasopharyngeal swabs between the first and third waves of COVID-19 in immunocompromised children. Each dot represents an individual RNA VL measure and the shape of them indicates the wave (first wave: empty boxes; third wave: solid circles). Curves represent the trajectory of RNA VL since SARS-CoV-2 diagnosis for each wave of COVID-19 (first wave: continuous line; third wave: dashed line) estimated by linear mixed-effects model

analyzed the level of RNA VL in each group per week over the first three weeks since diagnosis (Table 2). Notably, at the end of this period, RNA VL persisted around 10,000 copies/mL in both groups. By the end of the follow-up period, RNA VL level fell below de limit of quantification—considered as RNA clearance—at days 51 and 39 since diagnosis in G1 and G2, respectively. In an independent analysis performed by Kaplan–Meier model, the 50% of the patients reached undetectable levels of RNA VL at day 48 and 34 since diagnosis in G1 and G2, respectively (Fig. 2). However, this difference was not statistically significant ($P = 0.051$).

Finally, the correlation between RNA VL values and C_T values of the N gene—determined by qualitative test for SARS-CoV-2 diagnosis—was evaluated. Over a total of 186 nasopharyngeal swabs samples analyzed, both RNA VL and C_T measures correlated significantly ($r = -0.87$, $P < 0.001$).

Discussion

In this retrospective cohort study, immunocompromised pediatric patients with acquired SARS-CoV-2 infection during the first and third waves of COVID-19 in Argentina exhibited a very similar pattern of RNA VL throughout the course of infection. We also observed by studying each patient longitudinally that SARS-CoV-2 RNA persisted at high levels for more than three weeks in both waves. To our knowledge, this is the first study describing the natural history of SARS-CoV-2 RNA VL in immunocompromised pediatric patients, even comparing the RNA VL dynamics across different waves of COVID-19.

SARS-CoV-2 RNA VL data in immunocompromised children is scarce. Similar to findings from immunocompromised children study conducted over the first two waves of COVID-19 (from March 2020 to March 2021) in United States by Dolan et al. [18], we observed a prolonged viral persistence of SARS-CoV-2 in our cohort. In fact, Dolan and colleagues found that the median time from positive to negative real time RT-PCR was six weeks, while in our study, the time to achieve RNA levels below the limit of quantification was greater than five weeks. Comparing viral persistence between both waves studied, we observed that time to reach non-quantifiable levels of RNA VL was longer during the first wave—attributed to ancestral infections—compared to the third one—attributed to Omicron infections. However, this difference was not statistically significant not only by LME but also by Kaplan–Meier analysis. In fact, most of the patients in both waves (62% and 73% in the first and third waves, respectively) had sustained quantifiable RNA VL levels beyond fourth week since SARS-CoV-2 diagnosis. Contrary to our findings, a preliminary preprint reported by Hay et al. suggested that the duration of viral shedding may be shorter and clearance more rapid in patients infected with Omicron variant in comparison to those infected with previous variants [19]. However, in a later report of 1,280 individuals, the same authors found a similar clearance time between Omicron (6.2 days) and Delta (7.6 days) infection in vaccinated individuals, but they were shorter than non-Delta and non-Omicron infections in unvaccinated individuals [20]. Also, recent studies have found no difference regardless of SARS-CoV-2 viral shedding between Omicron and Delta variants [21, 22]. Nevertheless, most of the subjects included in those studies were immunocompetent and vaccinated, whereas in our cohort, most of the patients were unvaccinated (90%). Surprisingly, patients vaccinated against SARS-CoV-2 ($n = 3$) showed a viral RNA persistence beyond

Table 2 RNA viral load level per week during the first four weeks since SARS-CoV-2 diagnosis

Week*	$\log_{10}$ copies/mL, media (95% CI)	
	Group 1	Group 2
1	4.93 (4.14–5.72)	5.18 (4.42–5.94)
2	4.51 (3.78–5.25)	4.56 (3.88–5.25)
3	4.10 (3.40–4.80)	3.95 (3.31–4.59)
4	3.69 (2.99–4.38)	3.33 (2.71–3.96)

*Weeks from the first positive result for SARS-CoV-2 detection by RT-PCR

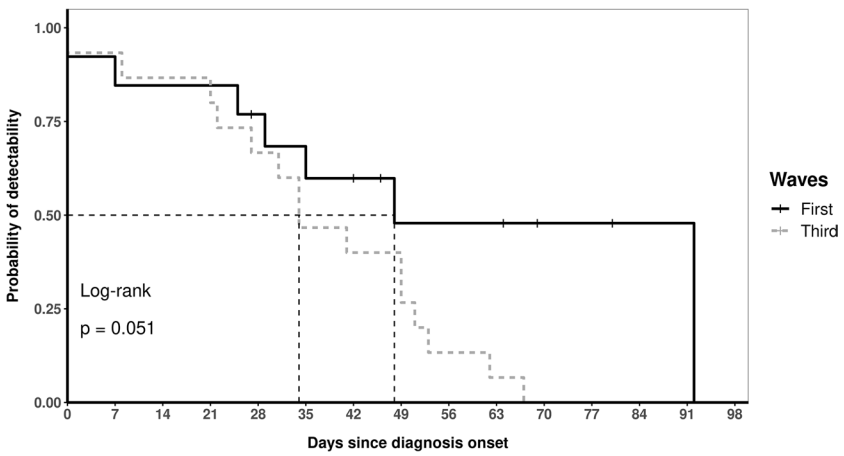

Fig. 2 Kaplan–Meier survival curves for RNA viral load in all children included in the study ($n = 28$) and grouped according to the wave of COVID-19

40 days (since SARS-CoV-2 diagnosis) similarly to the unvaccinated ones. Due to the small number of vaccinated patients in our cohort, we cannot draw conclusions on this matter. In recent literature, numerous studies have demonstrated that vaccines accelerate viral clearance in immunocompetent individuals [23–25]. However, there is a significant gap in the research when it comes to the effects of vaccination on viral clearance time in immunocompromised individuals. It is therefore important to investigate whether there is a difference in the viral clearance time between vaccinated and unvaccinated immunocompromised patients.

Over the first two waves of COVID-19, there have been reports that immunocompromised pediatric patients were at no increased risk of severe COVID-19 [26–28]. In contrast, other authors found that persons with immunocompromising conditions are at elevated risk of severe outcomes, hospitalization, and death from COVID-19 [29]. In our cohort of immunocompromised children, 89% had asymptomatic or mild disease, with no difference between the first and third waves. In addition, we do not find an association between RNA VL and disease severity in concordance with a large pediatric cohort study reported by Ochoa et al. [30]. Therefore, our results reinforce the notion that there is not a direct association between these two variables.

An important strength of this work is that RNA VL measures were performed by a single methodology based on an in-house quantitative real time RT-PCR, which was validated following the Clinical Laboratory Improvement Amendments—CLIA—standards reviewed by Burd et al. [31]. In this way, we used a standard curve to quantify the amount of virus expressed in RNA copies per milliliter, instead of using naive C_T values, which avoid a misunderstanding of RNA VL kinetics for comparison across different amplification runs, as described by Han et al. [32]. By contrast, the main limitation of this study was that the small size of our cohort precluded analyses to account for factors that may influence RNA VL dynamics (e.g., type of immunosuppression condition) and increased the risk of a type II error. Also, the characterization of SARS-CoV-2 viral variant could not be performed in our cohort. However, as was mentioned, the ancestral and Omicron variants were highly predominant over the period of study in the first and third waves, respectively, based on local epidemiological reports [15, 16]. Lastly, we did not test for the presence of infectious virus, and our findings are based on RNA VL values obtained from a standardized quantitative method.

In conclusion, our results suggest no difference between the Omicron and ancestral infections on the dynamics of SARS-CoV-2 RNA VL in immunocompromised pediatric patients and add evidence in favor of the higher viral persistence in this population. Also, our observations support the use of test-based, rather than time-based, protocols for defining the duration of isolation in immunocompromised individuals to limit the spread of SARS-CoV-2.

Author contribution MM conceptualized and designed the study, developed and performed virological measurements, and drafted the initial manuscript and reviewed it. AM conceptualized and designed the study and drafted the initial manuscript and reviewed it. MDG and MFF developed and performed virological measurements and reviewed the manuscript. AA, MP, RB, SR, and DB collected and analyzed clinical data and critically reviewed the manuscript for important intellectual content. MR performed statistical analysis and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Institutional Review Board (Comité Revisor y de Ética en la Investigación, Hospital de Pediatría "Prof. Dr. Juan P. Garrahan" Protocol No. 1359), and written informed consent was obtained from parents or legal guardians.

Competing interests The authors declare no competing interests.

References

1. Marks M, Millat-Martinez P, Ouchi D, Roberts CH, Alemany A, Corbacho-Monné M, Ubals M, Tobias A, Tebé C, Ballana E et al (2021) Transmission of COVID-19 in 282 clusters in Catalonia, Spain: a cohort study. Lancet Infect Dis 21:629–636. https://doi.org/10.1016/S1473-3099(20)30985-3
2. Singanayagam A, Patel M, Charlett A, Bernal JL, Saliba V, Ellis J, Ladhani S, Zambon M, Gopal R (2020) Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Eurosurveillance 25:1. https://doi.org/10.2807/1560-7917.ES.2020.25.32.2001483
3. European Centre for Disease Prevention and Control. Guidance on ending the isolation period for people with COVID. https://www.ecdc.europa.eu/en/publications-data/covid-19-guidance-discharge-and-ending-isolation. Accessed 15 Dec 2022
4. The Royal College of Paediatrics and Child Health. Guidance: paediatric multisystem inflammatory syndrome temporally associated with COVID-19. (2020). https://www.rcpch.ac.uk/resources/paediatric-multisystem-inflammatory-syndrome-temporally-associated-covid-19-pims-guidance. Accessed 25 Jan 2021
5. Nakajima Y, Ogai A, Furukawa K, Arai R, Anan R, Nakano Y, Kurihara Y, Shimizu H, Misaki T, Okabe N (2021) Prolonged viral shedding of SARS-CoV-2 in an immunocompromised patient. J Infect Chemother 27:387–389. https://doi.org/10.1016/j.jiac.2020.12.001
6. Baang JH, Smith C, Mirabelli C, Valesano AL, Manthei DM, Bachman MA, Wobus CE, Adams M, Washer L, Martin ET et al (2021) Prolonged severe acute respiratory syndrome coronavirus 2 replication in an immunocompromised patient. J Infect Dis 223:23–27. https://doi.org/10.1093/infdis/jiaa666
7. Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, Solomon IH, Kuo H-H, Boucau J, Bowman K et al (2020)

Persistence and evolution of SARS-CoV-2 in an immunocompromised host. N Engl J Med 383:2291–2293. https://doi.org/10.1056/NEJMc2031364
8. Niyonkuru M, Pedersen RM, Assing K, Andersen TE, Skov MN, Johansen IS, Madsen LW (2021) Prolonged viral shedding of SARS-CoV-2 in two immunocompromised patients, a case report. BMC Infect Dis 21:1–5. https://doi.org/10.1186/S12879-021-06429-5/FIGURES/1
9. Avanzato VA, Matson MJ, Seifert SN, Pryce R, Williamson BN, Anzick SL, Barbian K, Judson SD, Fischer ER, Martens C et al (2020) Case study: prolonged infectious SARS-CoV-2 shedding from an asymptomatic immunocompromised individual with cancer. Cell 183:1901-1912.e9. https://doi.org/10.1016/j.cell.2020.10.049
10. Morishita M, Suzuki M, Matsunaga A, Ishizhima K, Yamamoto T, Kuroda Y, Kanno T, Tsujimoto Y, Ishida A, Hashimoto M et al (2022) Prolonged SARS-CoV-2 infection associated with long-term corticosteroid use in a patient with impaired B cell immunity. J Infect Chemother 28:971. https://doi.org/10.1016/J.JIAC.2022.02.006
11. Sepulcri C, Dentone C, Mikulska M, Bruzzone B, Lai A, Fenoglio D, Bozzano F, Bergna A, Parodi A, Altosole T et al (2021) The longest persistence of viable SARS-CoV-2 with recurrence of viremia and relapsing symptomatic COVID-19 in an immunocompromised patient—a case study. Open Forum Infect Dis 8(11):ofab217. https://doi.org/10.1093/OFID/OFAB217
12. Quaranta EG, Fusaro A, Giussani E, D'Amico V, Varotto M, Pagliari M, Giordani MT, Zoppelletto M, Merola F, Antico A et al (2022) SARS-CoV-2 intra-host evolution during prolonged infection in an immunocompromised patient. Int J Infect Dis 122:444–448. https://doi.org/10.1016/J.IJID.2022.06.023
13. Hill V, Plessis L du, Peacock TP, Aggarwal D, Colquhoun R, Carabelli AM, Ellaby N, Gallagher E, Groves N, Jackson B et al (2022) The origins and molecular evolution of SARS-CoV-2 lineage B.1.1.7 in the UK. Virus Evol 8(2):veac080. https://doi.org/10.1093/ve/veac119
14. Li P, de Vries AC, Kamar N, Peppelenbosch MP, Pan Q (2022) Monitoring and managing SARS-CoV-2 evolution in immunocompromised populations. Lancet Microbe 3:e325. https://doi.org/10.1016/S2666-5247(22)00061-1
15. Proyecto Argentino Interinstitucional de genómica de SARS-CoV-2 -PAIS-. Reporte Nro 3 - CABA y Provincia de Buenos Aires, 31 de julio de 2020. http://pais.qb.fcen.uba.ar/content-report e3.php. Accessed 29 Oct 2022
16. Ministerio de Salud de Argentina. COVID-19. Situación de nuevas variantes SARS-CoV-2 en Argentina SE11/2022. (2022). https://www.argentina.gob.ar/sites/default/files/2022/03/03-22-vigilancia-genomica-se11.pdf. Accessed 29 Oct 2022
17. Golemba MD, Moragas M, Fernández MF, Borgnia D, Ruhle M, Palladino M, Arias AP, Ruvinsky S, Bologna R, Mangano A (in press) (2022) Comparación de la carga viral de SARS-CoV-2 en niños asintomáticos y sintomáticos atendidos en un hospital pediátrico público de referencia en Argentina. Rev Argent Microbiol. https://doi.org/10.1016/j.ram.2022.09.002
18. Dolan SA, Mulcahy Levy J, Moss A, Pearce K, Butler M, Jung S, Dominguez SR, Mwangi E, Maloney K, Rao S (2021) SARS-CoV-2 persistence in immunocompromised children. Pediatr Blood Cancer 68:e29277. https://doi.org/10.1002/PBC.29277
19. Hay JA, Kissler SM, Fauver JR, Mack C, Tai CG, Samant RM, Connelly S, Anderson DJ, Khullar G, MacKay M, et al. (Preprint) (2022) Viral dynamics and duration of PCR positivity of the SARS-CoV-2 Omicron variant. *medRxiv* 2022.01.13.22269257. https://doi.org/10.1101/2022.01.13.22269257
20. Hay JA, Kissler SM, Fauver JR, Mack C, Tai CG, Samant RM, Connolly S, Anderson DJ, Khullar G, MacKay M et al (2022) Quantifying the impact of immune history and variant on SARS-CoV-2 viral kinetics and infection rebound: a retrospective cohort study. Elife 11:81849. https://doi.org/10.7554/ELIFE.81849
21. Boucau J, Marino C, Regan J, Uddin R, Choudhary MC, Flynn JP, Chen G, Stuckwisch AM, Mathews J, Liew MY et al (2022) Duration of shedding of culturable virus in SARS-CoV-2 Omicron (BA.1) infection. N Engl J Med 387:275–277. https://doi.org/10.1056/NEJMc2202092
22. Bouton TC, Atarere J, Turcinovic J, Seitz S, Sher-Jan C, Gilbert M, White L, Zhou Z, Hossain MM, Overbeck V et al (2022) Viral dynamics of Omicron and Delta severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants with implications for timing of release from isolation: a longitudinal cohort study. Clin Infect Dis. https://doi.org/10.1093/CID/CIAC510
23. Singanayagam A, Hakki S, Dunning J, Madon KJ, Crone MA, Koycheva A, Derqui-Fernandez N, Barnett JL, Whitfield MG, Varro R et al (2022) 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 22:183–195. https://doi.org/10.1016/S1473-3099(21)00648-4
24. Kissler SM, Fauver JR, Mack C, Tai CG, Breban MI, Watkins AE, Samant RM, Anderson DJ, Metti J, Khullar G et al (2021) Viral dynamics of SARS-CoV-2 variants in vaccinated and unvaccinated persons. N Engl J Med 385:2489–2491. https://doi.org/10.1056/NEJMC2102507
25. Puhach O, Adea K, Hulo N, Sattonnet P, Genecand C, Iten A, Jacquérioz F, Kaiser L, Vetter P, Eckerle I et al (2022) Infectious viral load in unvaccinated and vaccinated individuals infected with ancestral, Delta or Omicron SARS-CoV-2. Nat Med 28:1491–1500. https://doi.org/10.1038/s41591-022-01816-0
26. Chappell H, Patel R, Driessens C, Tarr AW, Irving WL, Tighe PJ, Jackson HJ, Harvey-Cowlishaw T, Mills L, Shaunak M et al (2022) Immunocompromised children and young people are at no increased risk of severe COVID-19. J Infect 84:31–39. https://doi.org/10.1016/J.JINF.2021.11.005
27. Minotti C, Tirelli F, Barbieri E, Giaquinto C, Donà D (2020) How is immunosuppressive status affecting children and adults in SARS-CoV-2 infection? A systematic review. J Infect 81:e61–e66. https://doi.org/10.1016/J.JINF.2020.04.026
28. Nicastro E, Verdoni L, Bettini LR, Zuin G, Balduzzi A, Montini G, Biondi A, D'Antiga L (2021) COVID-19 in immunosuppressed children. Front Pediatr 9:225. https://doi.org/10.3389/FPED.2021.629240/BIBTEX
29. Jakharia N, Subramanian AK, Shapiro AE (2022) COVID-19 in the immunocompromised host, including people with human immunodeficiency virus. Infect Dis Clin North Am 36:397. https://doi.org/10.1016/J.IDC.2022.01.006
30. Ochoa V, Díaz FE, Ramirez E, Fentini MC, Carobene M, Geffner J, Arruvito L, Remes Lenicov F, Adamczyk A, Azzolina S et al (2022) Infants younger than 6 months infected with SARS-CoV-2 show the highest respiratory viral loads. J Infect Dis 225:392–395. https://doi.org/10.1093/INFDIS/JIAB577
31. Burd EM (2010) Validation of laboratory-developed molecular assays for infectious diseases. Clin Microbiol Rev 23:550–576. https://doi.org/10.1128/CMR.00074-09
32. Han MS, Byun JH, Cho Y, Rim JH. (2020) RT-PCR for SARS-CoV-2: quantitative versus qualitative. Lancet Infect Dis 21(2):165. https://doi.org/10.1016/S1473-3099(20)30424-2